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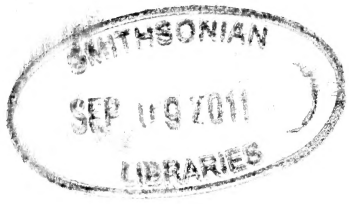

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EDAPHIC ECOLOGY AND GENETICS OF THE GABBRO-ENDEMIC SHRUB *CEANOTHUS RODERICKII* (RHAMNACEAE)

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ABSTRACT

Edaphic-endemic plant taxa are often interpreted as recently derived entities that evolved in situ, with genetic divergence driven by substrate specialization. However, little is known about the evolution of specific edaphic-endemic taxa, particularly the role that soil conditions may play in their initial divergence and continued persistence. Our study focuses on *Ceanothus roderickii*, a strict specialist on soils derived from a single outcrop of the geological material gabbro located in southwestern El Dorado County, California. In order to elucidate the evolutionary history of *C. roderickii* we sequenced the third intron of the low-copy nuclear gene nitrate reductase (NIA) for individuals representing four populations of *C. roderickii* and a wide taxonomic and geographic sampling of closely related plants, including 37 populations of *Ceanothus cuneatus* and a single representative from 16 other taxa. Analysis of NIA shows that *C. roderickii* is closely related to *C. cuneatus* var. *cuneatus*, a widely distributed taxon found on a diversity of soils. *Ceanothus cuneatus* var. *cuneatus* is paraphyletic and comprises two major geographic groups, one coastal and one interior, the latter containing *C. roderickii*. Thirteen soil chemistry variables were assayed in 42 populations of *C. cuneatus* representing the wide geographic range of this species, and in 10 populations of *C. roderickii*. Analysis of these data indicates that evolution of *C. roderickii* was associated with specialization to nutrient-deficient forms of gabbro-derived soil. Soil chemistry associations of *C. cuneatus* var. *cuneatus* and *C. roderickii* are most divergent where the species come into close contact on gabbro, with *C. cuneatus* var. *cuneatus* occupying comparatively nutrient-rich forms of gabbro-derived soils, a result that is consistent with reinforcement.

Key Words: *Ceanothus*, edaphic, evolution, gabbro, NIA, Pine Hill intrusive complex.

Edaphic factors—those pertaining to the substrate or soil—have long been interpreted as potential drivers of plant diversification (Stebbins 1942; Kruckeberg 1986; Rajakaruna 2004). This idea derives from the strong association of many so-called 'edaphic endemic' taxa with particular soil or substrate conditions (Mason 1946; Gankin and Major 1964; Kruckeberg 1986, 2002). In California, for example, approximately 10% of native vascular plants at the level of species and below are endemic to soils derived from serpentinite parent material (Kruckeberg 1986; Hickman 1993). Edaphic endemics are often classified either as relicts (paleoendemics) or as recently derived entities (neoendemics) that evolved in situ, with substrate specialization accompanying genetic divergence (Raven and Axelrod 1978). Recent work by Baldwin (2005) provided the first phylogenetic evidence for recent divergence of an edaphic endemic taxon, discovering that the serpentinite-endemic herb *Layia discoidea* D. D. Keck "budded off" from within a less specialized species less than 1 mya. It is not clear, however, whether this pattern is common to the large number of other edaphic endemics in California and elsewhere. By combining detailed genetic surveys with analyses of edaphic conditions experienced by edaphic endemics and their close relatives, it may be possible to discern general trends in the evolution of edaphic endemics, and how these trends relate to soil conditions. Here we focus on the *Cerastes* subgenus of *Ceanothus*, which contains 10 edaphic-endemic taxa restricted to California and Baja California, Mexico (Table 1). Our goal is to discern the evolutionary history of a single species of edaphic endemic *Cerastes*, and relate this history to the substrate conditions experienced by this taxon and its closest relatives.

In the Sierra Nevada foothills of western El Dorado County, California, soils weathered from mafic rocks of the Pine Hill intrusive complex (~100 km^2, Fig. 1; Springer 1971) support approximately 600 vascular plant species (Wilson et al. 2009, Appendix 1), representing more than 10% of the California flora (5867 species; Hickman 1993), including several endemics and taxa of limited or disjunct distribution (Wilson 1986; Hunter and Horenstein 1991; Wilson et al. 2009). Endemics of the Pine Hill intrusive complex include *Ceanothus roderickii* W. Knight, *Fremontodendron californicum* (Torr.) Coville subsp. *decumbens* (R. M. Lloyd) Munz, *Galium californicum* Hook. & Arn. subsp. *sierrae* Dempster & Stebbins, and *Wyethia reticulata* Greene. The first three of these plants are federally-listed Endangered Species (USFWS 1996).

The Pine Hill intrusive complex is composed primarily of the rock gabbro, with minor

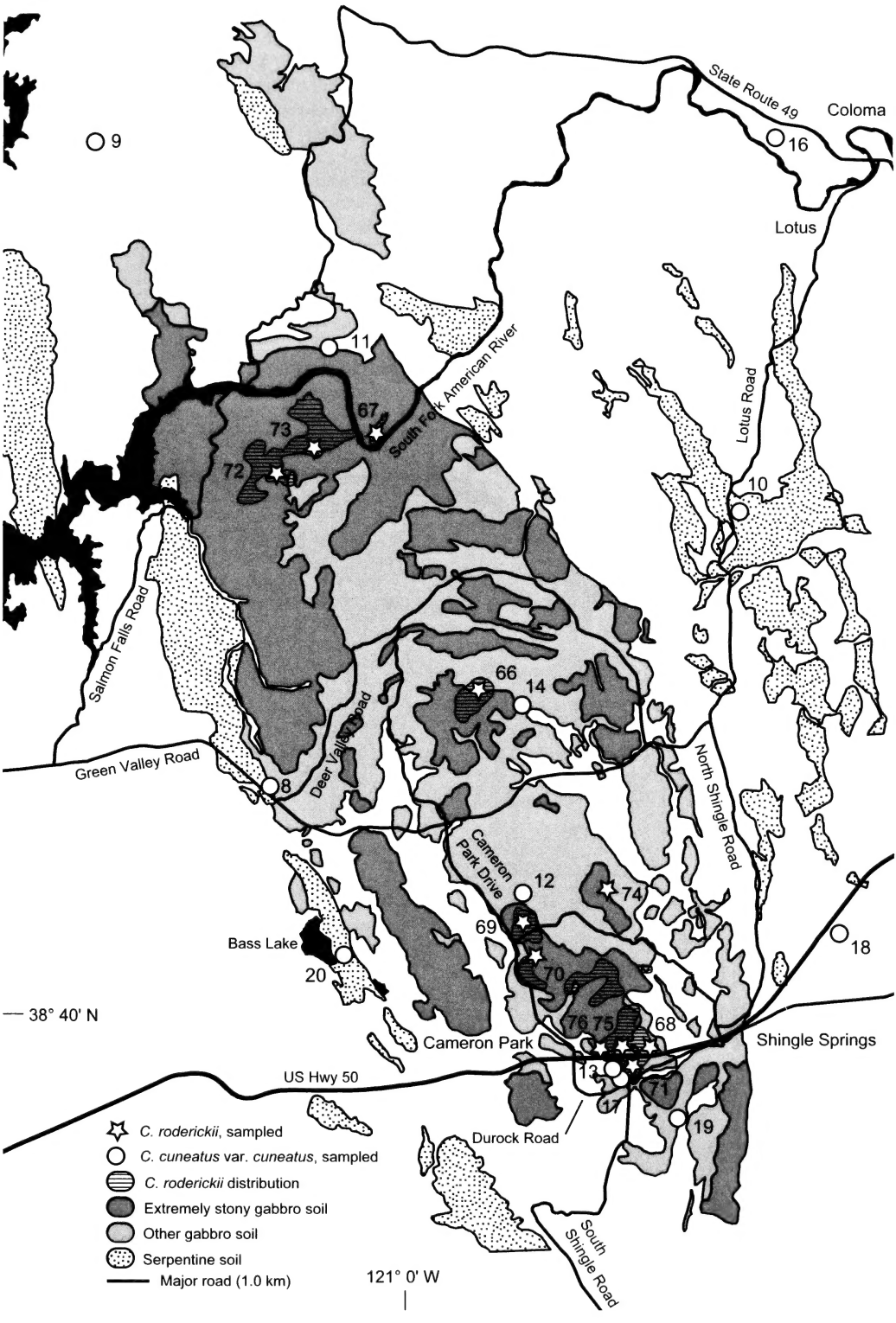
TABLE 1. *CEANOTHUS*, SUBGENUS *CERASTES* TAXA. Taxon = taxa from Fross and Wilken (2006). Sampled = populations sampled for genetic and/or soil analyses (Appendix 1). Geographic distribution = distribution of taxa in North America (North CA: region of CA from the latitude of Point Conception, north; South CA: region of CA from the latitude of Point Conception, south; BC, Mexico: Mexican state of Baja California). Soil = geological parent material(s) for soils on which taxon occurs (Fross & Wilken 2006). [a] 25 populations of *C. cuneatus* var. *cuneatus* sampled for genetics and soil, 8 for genetics only, and 13 for soil only. [b] Three populations of *C. roderickii* sampled for genetics and soil, 1 for genetics only, and 7 for soil only. [c] Also found on gabbro-derived soils. [d] Parent material classified as metavolcanic ("Mzv") by Jennings (1977).

Taxon	Sampled	Geographic distribution	Soil
Ceanothus arcuatus McMinn	0	North CA	various
C. bolensis S. Boyd & J.E. Keeley	0	BC, Mexico	basalt
C. crassifolius Torr. var. *crassifolius*	0	South CA; BC, Mexico	various
C. crassifolius Torr. var. *planus* Abrams	0	South CA	various
C. cuneatus Nutt. var. *cuneatus*	46[a]	West US; BC, Mexico	various
C. cuneatus Nutt. var. *dubius* J.T. Howell	1	North CA	various
C. cuneatus Nutt. var. *fascicularis* (McMinn) Hoover	1	North CA	various
C. cuneatus Nutt. var. *ramulosus* Greene	1	North CA	various
C. cuneatus Nutt. var. *rigidus* (Nutt.) Hoover	1	North CA	various
C. divergens Parry subsp. *confusus* (J.T. Howell) Abrams	1	North CA	various
C. divergens Parry subsp. *divergens*	0	North CA	various
C. divergens Parry subsp. *occidentalis* (McMinn) Abrams	1	North CA	various
C. ferrisiae McMinn	1	North CA	serpentinite
C. fresnensis Abrams	1	North CA	various
C. gloriosus J.T. Howell var. *exaltatus* J.T. Howell	1	North CA	various
C. gloriosus J.T. Howell var. *gloriosus*	1	North CA	various
C. gloriosus J.T. Howell var. *porrectus* J.T Howell	1	North CA	granite
C. jepsonii Greene var. *albiflorus* J.T. Howell	1	North CA	serpentinite
C. jepsonii Greene var. *jepsonii*	1	North CA	serpentinite
C. maritimus Hoover	1	North CA	various
C. masonii McMinn	1	North CA	various
C. megacarpus Nutt. var. *insularis* (Eastw.) Munz	0	South CA	various
C. megacarpus Nutt. var. *megacarpus*	0	South CA	various
C. ophiochilus S. Boyd, T.S. Ross & Arnseth	0	South CA	pyroxenite[c]
C. otayensis McMinn	0	South CA; BC, Mexico	basalt[d]
C. pauciflorus DC.	0	Mexico	various
C. perplexans Trel.	0	South CA; BC, Mexico	various
C. pinetorum Coville	1	North CA	various
C. prostratus Benth.	1	North CA	various
Ceanothus pumilus Greene	1	North CA	serpentinite
C. purpureus Jeps.	1	North CA	volcanic
C. roderickii W. Knight	11[b]	North CA	gabbro
C. sonomensis J.T. Howell	1	North CA	various
C. verrucosus Nutt.	0	South CA; BC, Mexico	various
C. vestitus Greene	0	West US; Mexico	various

amounts of pyroxenite and diorite (Springer 1980; hereafter referred to collectively as "gabbro"), which weather to form reddish-brown sandy loams with very stony to clayey variants (the Rescue Series; Rogers 1974). Gabbro contains less iron, Mg, and potentially plant-toxic transition elements (e.g., Cr, Co, Ni) than are found in ultramafic rocks such as serpentinite

→

FIG. 1. Sampling and soil map for the Pine Hill region, El Dorado Co., California. Polygons for gabbro or serpentinite derived soils adapted from GIS data layers in Soil Survey Geographic (SSURGO) database for El Dorado Area, California (U.S. Department of Agriculture, Natural Resources Conservation Service 2007). *Extremely stony gabbro soil*: shallow soils derived from gabbro parent material, corresponding to "Rescue extremely stony sandy loam" (Rogers 1974, RgE2). *Other gabbro soil*: deeper soils derived from gabbro parent



material, corresponding to "Rescue sandy loam" (Rogers 1974, ReC, ReB, & ReD) and "Rescue very stony sandy loam" (Rogers 1974, RfC, RfD, & RfE). *Serpentine soil*: very shallow, rocky soils derived from serpentinite parent material, corresponding to 'Serpentine rock land' (Rogers 1974, SaF). *Ceanothus roderickii* distribution adapted from Hinshaw (2008) in consultation with G. Hinshaw and L. Fety (March, 2010). Sampling locations indicated by stars or open circles (Table 2).

(Alexander 1993, unpublished). As a result, soils derived from gabbro usually contain elevated levels of Mg relative to soils derived from less mafic rocks such as diorite, but have lower Ca to Mg ratios than soils weathered from ultramafic rocks such as serpentinite (Goldhaber et al. 2009).

Endemism on the Pine Hill intrusive complex, as well as the presence of species normally restricted to serpentinite-derived soils, has been attributed to the similar properties of gabbro and serpentinite rock (Wilson 1986). However, analyses of soils from the Pine Hill intrusive complex have not identified soil parameters that predict plant distributions (Hunter and Horenstein 1991; Alexander, unpublished), leading Hunter and Horenstein (1991) to conclude that endemism on these soils may be attributed to the island-like topographic position of the complex in the otherwise low-lying foothills of the central Sierra Nevada. However, these results may be confounded by plant demography, especially in the case of *C. roderickii*, which is dependant on fire for significant recruitment (Boyd 2007).

Ceanothus roderickii is a member of the *Cerastes* subgenus of *Ceanothus*, a group of 24 species that is almost entirely restricted to the California Floristic Province (CFP) of western North America (Fross and Wilken 2006). Members of *Cerastes* possess a suite of morphological and physiological adaptations for drought resistance (Ackerly et al. 2006) and are associated with chaparral vegetation. However, the group is both morphologically and ecologically diverse, with an array of growth forms and a broad spectrum of habitat associations, sometimes including specialized edaphic ecology (McMinn 1942; Nobs 1963; Fross and Wilken 2006). *Ceanothus roderickii* is a decumbent shrub spreading horizontally via arching branches that usually root adventitiously when nodes contact soil (Knight 1968), a trait that allows this species to reproduce clonally during fire-free intervals when recruitment from the seed-bank is limited (Boyd 2007). A close relationship between *C. roderickii* and the widespread *Ceanothus cuneatus* Nutt. was proposed by Knight (1968). However, this author also speculated on the possibility of a close relationship between *C. roderickii* and *Ceanothus fresnensis* Abrams or *Ceanothus prostratus* Benth., the only other decumbent members of *Cerastes* known from the central Sierra Nevada.

Sequence data from nuclear ribosomal DNA suggest that *C. roderickii* is closely related to *C. cuneatus* var. *cuneatus* (Hardig et al. 2000). *Ceanothus cuneatus* is among the most widely distributed members of *Ceanothus*, occupying forest, woodland, and chaparral habitats at low to moderate elevations in far western North America from Baja California, Mexico to northwestern Oregon (Fig. 2), almost entirely within the CFP (Fig. 2). *Ceanothus cuneatus* comprises five varieties (Table 1), four of which are narrowly distributed (Fross and Wilken 2006). The most widely distributed variety, *C. cuneatus* var. *cuneatus*, is a characteristic component of chaparral and woodland communities in the foothills and mountains of the CFP and is known to grow on soils derived from a variety of geological parent materials (Fross and Wilken 2006; Table 1). *Ceanothus cuneatus* var. *cuneatus* is the only member of *Cerastes* other than *C. roderickii* known to occur in the Pine Hill region of El Dorado Co., California.

This study was designed to elucidate the evolutionary history of *C. roderickii* and relate this history to the substrate specificity of the taxon and its closest relatives. Specifically, this study aimed to 1) test the hypothesis that *C. roderickii* is most closely related to and possibly derived from within *C. cuneatus* var. *cuneatus*, 2) characterize the soil chemistry associations of *C. roderickii* relative to those of *C. cuneatus* var. *cuneatus*, and 3) identify specific chemical properties of *C. roderickii* soils that may have provided selective pressure during evolution of the species.

Materials and Methods

Genetic Sampling

Genetic sampling of *Ceanothus* populations was designed to represent the geographic range and edaphic tolerances of the focal taxa *C. roderickii* and *C. cuneatus* var. *cuneatus*, and to encompass related plants (Tables 1 and 2, Fig. 2, Appendix 1). DNA from 57 plants was studied, representing 22 of the approximately 35 *Cerastes* taxa (species, subspecies, and varieties) currently recognized (Table 1; Fross and Wilken 2006). All individuals sampled for the present work were collected by D. Burge, with the exception of a sample of *Ceanothus pinetorum* Coville obtained by D. Wilken (DHW 16736, Table 2, Appendix 1). Individuals from four populations of *C. roderickii* were sampled to represent the geographic distribution of this species (Table 2, Fig. 1, Appendix 1). Individuals from 33 populations of *C. cuneatus* var. *cuneatus* were sampled to represent the extensive geographic range of this taxon and the variety of edaphic conditions that it experiences over this area (Table 2, Fig. 2). Individuals representing populations of 20 additional *Cerastes* taxa were sampled for genetic analysis based on a large-scale phylogenetic study of *Ceanothus* (Burge et al. in press). In this large-scale study, which is based on more than 140 *Cerastes* populations from across the geographic range of the group, individuals included in the present study (Table 2, Appendix 1) form a

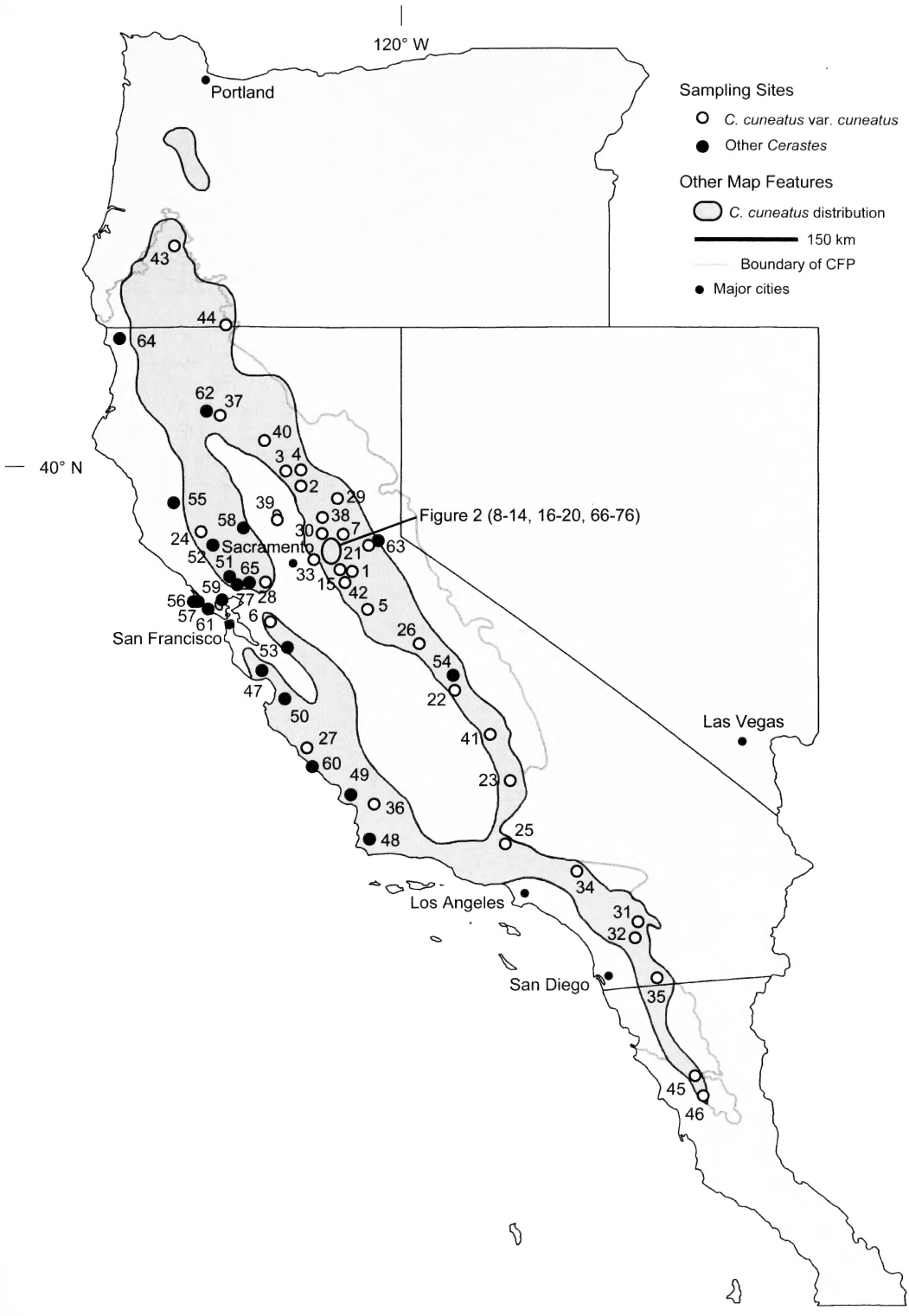


FIG. 2. Sampling map for western North America. Soil and/or genetic sampling locations indicated by open circles (Table 2). Global distribution of *C. cuneatus* indicated by gray shading (data provided by the participants of the Consortium of California Herbaria; March, 2010). Boundary of the California Floristic Province (CFP) adapted from Myers et al. (2000).

TABLE 2. GENETIC AND SOIL SAMPLING. Taxon = taxa from Fross and Wilken (2006). Map = numbering corresponds to Figs. 1 and 2. State, county & voucher = state and county for plant and/or soil sampling, and collection number for corresponding voucher specimen (Appendix 1); all collections by D. O. Burge (except DHW 16736, see Appendix 1), all voucher specimens deposited at DUKE. Soil = generalized geological parent material from which local soil is derived; interpreted based on Jennings (1977). GenBank = GenBank accession number(s) for NIA; –, no sequence data.[a]Soil chemistry data obtained. See Appendix 1 for additional data.

Taxon	Map	State, county & voucher	Soil	GenBank
C. cuneatus Nutt. var. *cuneatus*	1	CA; Amador; 1150a	metamorphic[a]	HM240329; HM240330
	2	CA; Butte; 1109a	granite[a]	—
	3	CA; Butte; 815a	volcanic[a]	—
	4	CA; Butte; 1078a	serpentinite[a]	HM240306; HM240307
	5	CA; Calaveras; 1149a	sedimentary[a]	HM240327; HM240328
	6	CA; Contra Costa; 916a	sedimentary[a]	HM240341; HM240342
	7	CA; El Dorado; 1011a	serpentinite[a]	—
	8	CA; El Dorado; 1024a	serpentinite[a]	—
	9	CA; El Dorado; 1074a	volcanic[a]	—
	10	CA; El Dorado; 1076a	serpentinite[a]	—
	11	CA; El Dorado; 1088a	gabbro[a]	—
	12	CA; El Dorado; 1089a	gabbro[a]	—
	13	CA; El Dorado; 1101a	gabbro[a]	—
	14	CA; El Dorado; 1116a	gabbro[a]	—
	15	CA; El Dorado; 1174a	sedimentary[a]	—
	16	CA; El Dorado; 1175a	granite[a]	—
	17	CA; El Dorado; 1023a	gabbro[a]	HM240296; HM240297
	18	CA; El Dorado; 1075a	metamorphic[a]	HM240302; HM240303
	19	CA; El Dorado; 1095a	gabbro[a]	HM240314; HM240315
	20	CA; El Dorado; 1110a	serpentinite[a]	HM240316
	21	CA; El Dorado; 1117a	volcanic[a]	HM240317; HM240318
	22	CA; Fresno; 1136a	metamorphic[a]	HM240323
	23	CA; Kern; 1132a	granite[a]	HM240319; HM240320
	24	CA; Lake; 1008a	sedimentary	HM240295
	25	CA; Los Angeles; 1071a	granite[a]	HM240301
	26	CA; Mariposa; 1140a	granite[a]	HM240324
	27	CA; Monterey; 858a	sedimentary	HM240338
	28	CA; Napa; 899a	sandstone	HM240339; HM240340
	29	CA; Nevada; 1084a	serpentinite[a]	HM240310
	30	CA; Placer; 1077a	metamorphic[a]	HM240304; HM240305
	31	CA; Riverside; 803a	granite[a]	—
	32	CA; Riverside; 982a	granite[a]	HM240344; HM240345
	33	CA; Sacramento; 1094a	sedimentary[a]	HM240313
	34	CA; San Bernardino; 1070a	sedimentary[a]	HM240300
	35	CA; San Diego; 984a	granite[a]	HM240346
	36	CA; San Luis Obispo; 959a	sedimentary	HM240343
	37	CA; Shasta; 1151a	metamorphic	HM240331; HM240332
	38	CA; Sierra; 1083a	serpentinite[a]	HM240308; HM240309
	39	CA; Sutter; 1093a	volcanic	HM240311; HM240312
	40	CA; Tehama; 1168a	volcanic[a]	HM240336
	41	CA; Tulare; 1134a	granite[a]	HM240321; HM240322

TABLE 2. Continued.

Taxon	Map	State, county & voucher	Soil	GenBank
	42	CA; Tuolumne; 1145a	serpentinite[a]	HM240325; HM240326
	43	OR; Douglas; 1161a	serpentinite	HM240333
	44	OR; Jackson; 1164a	volcanic	HM240334; HM240335
	45	Baja CA; N/A; 1030a	granite[a]	HM240298; HM240299
	46	Baja CA; N/A; 783a	metamorphic[a]	HM240337
C. cuneatus Nutt. var. *dubius* J.T. Howell	47	CA; Santa Cruz; 918a	sedimentary[a]	HM240347
C. cuneatus Nutt. var. *fascicularis* (McMinn) Hoover	48	CA; Santa Barbara; 871a	sand[a]	HM240348
C. cuneatus Nutt. var. *ramulosus* Greene	49	CA; San Luis Obispo; 847b	serpentinite[a]	HM240349
C. cuneatus Nutt. var. *rigidus* (Nutt.) Hoover	50	CA; Monterey; 891b	sedimentary[a]	HM240350; HM240351
C. divergens Parry subsp. *confusus* (J.T. Howell) Abrams	51	CA; Sonoma; 1003a	serpentinite	HM240352; HM240353
C. divergens Parry subsp. *occidentalis* (McMinn) Abrams	52	CA; Lake; 943a	volcanic	HM240354
C. ferrisiae McMinn	53	CA; Santa Clara; 834a	serpentinite	HM240355; HM240356
C. fresnensis Abrams	54	CA; Fresno; 1138a	granite	HM240357
C. gloriosus J.T. Howell var. *exaltatus* J.T. Howell	55	CA; Mendocino; 994a	sediment	HM240358; HM240359
C. gloriosus J.T. Howell var. *gloriosus*	56	CA; Marin; 908a	sand	HM240360; HM240361
C. gloriosus J.T. Howell var. *porrectus* J.T. Howell	57	CA; Marin; 907a	granite	HM240362; HM240363
C. jepsonii Greene var. *albiflorus* J.T. Howell	58	CA; Colusa; 997a	serpentinite	HM240364; HM240365
C. jepsonii Greene var. *jepsonii*	59	CA; Marin; 914a	serpentinite	HM240366
C. maritimus Hoover	60	CA; San Luis Obispo; 887a	sediment	HM240367
C. masonii McMinn	61	CA; Marin; 913a	sediment	HM240368
C. pinetorum Coville	62	CA; Trinity; DHW 16736	granite	HM240369; HM240370
C. prostratus Benth.	63	CA; El Dorado; 952a	metamorphic	HM240371
C. pumilus Greene	64	CA; Del Norte; 1156a	serpentinite	HM240372; HM240373
C. purpureus Jeps.	65	CA; Napa; 904a	volcanic	HM240374; HM240375
C. roderickii W. Knight	66	CA; El Dorado; 1080a	gabbro[a]	HM240376
	67	CA; El Dorado; 1087a	gabbro[a]	HM240377; HM240378
	68	CA; El Dorado; 1090a	gabbro[a]	—
	69	CA; El Dorado; 1096a	gabbro[a]	—
	70	CA; El Dorado; 1100a	gabbro[a]	—
	71	CA; El Dorado; 1102a	gabbro[a]	—
	72	CA; El Dorado; 1104a	gabbro[a]	—
	73	CA; El Dorado; 1105a	gabbro[a]	—
	74	CA; El Dorado; 1111	gabbro[a]	HM240379
	75	CA; El Dorado; 1171a	gabbro[a]	—
	76	CA; El Dorado; 824b	gabbro	HM240380
C. sonomensis J.T. Howell	77	CA; Sonoma; 895b	volcanic	HM240381

monophyletic group nested within *Cerastes* as a whole (Burge et al. in press). Voucher specimens were identified based on Fross and Wilken (2006). However, *Ceanothus masonii* McMinn, treated as part of *Ceanothus gloriosus* J. T. Howell by Fross and Wilken (2006) is recognized here.

Molecular Methods

Genomic DNA was extracted from fresh or silica-dried leaf and/or flower-bud tissue using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Polymerase chain reactions were performed using Qiagen *Taq* DNA Polymerase. Amplification was performed using an initial incubation at 94C for 10 min and 30 cycles of three-step PCR (1 min at 94C, 30 s at 55C, and 2 min at 72C), followed by final extension at 72C for 7 min. Primers NIA-3F and NIA-3R (Howarth and Baum 2002) were initially used to amplify the third intron of the low-copy nuclear gene nitrate reductase (NIA). Subsequent to amplification from representative *Ceanothus* species, NIA PCR products were cloned using the TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Non-degenerate primers (NIARHA-3F, AGGTGGAGGTTCTTAACCTTCTC; NIARHA-3R, GAACCAGCAATTGTTCATCATTCC) were designed based on the alignment of these cloned sequences. These primers have been used to amplify NIA from all members of *Cerastes* as well as members of the *Ceanothus* subgenus of *Ceanothus*. Analysis of NIA sequences from representative *Ceanothus* individuals (Burge et al. in press) demonstrated that these primers always amplify a single orthologous copy of NIA, which is frequently represented by two sequence types (putative alleles) that vary in length and base composition. As a result of this heterogeneity, cloning of NIA was required for most plants. Subsequent to initial primer design, cloning was carried out using the pGEM-T Easy Vector kit (ProMega, Madison, WI) according to the manufacturer's instructions. NIA inserts were amplified directly from 4–10 large positive colonies using the non-degenerate primers and PCR protocol described above. Excess primer and dNTPs were removed using exonuclease I (New England Biolabs, Ipswich, MA [NEB]; 0.2 units/μl PCR product) and antarctic phosphatase (NEB; 1.0 unit/μl PCR product) incubated for 15 min at 37C followed by 15 min at 80C. For sequencing, Big Dye chemistry (Applied Biosystems, Foster City, CA) was utilized according to manufacturer instructions. Sequences were determined on an Applied Biosystems 3100 Genetic Analyzer at the Duke University Institute for Genome Science and Policy Sequencing and Genetic Analysis Facility.

Phylogenetic Analysis

DNA sequences were assembled and edited using the program Sequencher 4.1 (Gene Codes Corporation). Sequences were aligned using MUSCLE (Edgar 2004) under the default settings, with minor adjustments made manually. For each individual plant (Table 2), sequence variation was assessed based on an alignment of cloned NIA sequences (hereafter "isolates") obtained from that plant. Twenty-four plants yielded pools of identical isolates, 30 contained two different types of NIA sequence (hereafter "sequence types"), and three were represented by a single successfully cloned isolate (Table 2). For plants with two sequence types, a single isolate representing each type was selected randomly for subsequent analysis (Table 2). For plants with one sequence type, a single isolate was selected at random from the pool of isolates for that plant (Table 2). A total of 87 isolates were selected for analysis and aligned and edited as described above. Two ambiguously-aligned regions were excluded from analysis (see below). Edited sequences were deposited in GenBank (Table 2, Appendix 1).

Phylogenetic analyses under the Bayesian criterion were conducted using the best-fit model of evolution from Akaike information criterion (AIC) output of the program MrModeltest v2 (Nylander 2004). Sampling of trees was performed using the program MrBayes 3 (Ronquist and Huelsenbeck 2003). Three separate runs of 5,000,000 MCMC generations were performed using one heated and three cold chains, sampling every 1000 generations. Independent chains were inspected for convergence (standard deviation of split frequencies nearing 0.001). Log-likelihood for the sampled tree was plotted and examined in Microsoft Excel. Sampled trees from the burn-in period (Ronquist and Huelsenbeck 2003) were identified and eliminated. A consensus phylogram for each independent run was constructed based on the post-burnin sample of trees using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). Trees from each of the three independent runs were compared to verify the similarity of the results. The final Bayesian consensus tree was manually rooted based on results from an expanded phylogenetic study of *Ceanothus* (Burge et al. in press).

Statistical parsimony (Templeton et al. 1992), as implemented in the program TCS (Clement et al. 2000), was used to reconstruct a gene genealogy for NIA based on the alignment described above. Statistical parsimony is a network-based method that accommodates reticulate relationships such as those that result from recombination, and therefore has advantages over traditional tree building methods such as parsimony, neighbor-joining, and maximum like-

lihood when considering population-level relationships (Clement et al. 2000). Analyses were conducted under default settings of the TCS program. The network output by TCS was adjusted manually in order to facilitate interpretation. The network was examined visually for loops (ambiguities) representing potential reticulate relationships among NIA isolates (Clement et al. 2000).

Utilization of Highly-Variable Regions

Initial inspection of NIA alignments revealed the presence of two highly-variable AT-rich regions. In the initial alignment of NIA isolates (see Results) the first variable region begins at position 136 (5′) and ends at position 152 (3′), with a maximum un-aligned length of 16 bases. The second region begins at position 401 (5′) and ends at position 448 (3′), with a maximum un-aligned length of 45 bases. Due to ambiguity inherent in aligning such regions, they were excluded from phylogenetic analysis, as described above. Initial inspection, however, showed that sequence variation in these regions corresponds with relationships implied by phylogenetic analysis of the remaining sequence data. Due to its less ambiguous alignment, the first variable region was focused upon for subsequent work. This highly-variable region was treated as a single character and unique "motif types" identified based on the exact sequence of the region. Each of the 87 NIA isolates was binned according to motif type. In a hypothetical example of this process, isolates from a four-base-pair-long highly-variable region with sequences of ATTT, AATT, and AAAT would represent three separate motif types. Following reconstruction of phylogenetic trees based on an alignment that excluded both of the highly-variable regions, motif type was mapped onto trees and used to help identify natural groups.

Soil Sampling

Fifty-two soils samples, representing 42 populations of *C. cuneatus* (including all five varieties of this species) and 10 populations of *C. roderickii*, were subjected to chemical analysis (Tables 1 and 2). Thirty-two of the 54 samples correspond to populations included in the genetic analysis (Tables 1 and 2). Sampling of soil was carried out in April and May, 2009. At each site, one liter of soil was collected by consolidation of sub-samples taken within the rooting zone of three plants growing in a 5 m^2 area. Sub-samples were collected using a garden trowel with a steel blade, excavating to a depth of at most 10 cm, depending on the depth of the soil profile. Soils were air-dried and returned to Duke University for storage and preparation.

Soil Chemistry Assays

Soil chemistry analyses were carried out by the Texas A&M University Soil, Water, and Forage Testing Laboratory. Samples were passed through a 2 mm sieve prior to analysis. Major nutrients (P, K, Ca, Mg, S) and sodium were extracted using the Mehlich III extractant (Mehlich 1978, 1984) and determined by inductively coupled plasma mass-spectroscopy (ICP). Micronutrients (Cu, Fe, Mn, and Zn) were extracted using a modified DPTA solution (Lindsay and Norvell 1978), and determined by ICP. Soil pH was determined in a 1:2 soil:deionized water extract (Schofield and Taylor 1955). Electrical conductivity (a proxy for soluble salts) was determined in a 1:2 soil:deionized water extract using a soil conductivity probe (Rhoades 1982). Finally, nitrate (NO_3^-) was extracted in 1 M KCl solution, reduced to nitrite (NO_2^-) using a cadmium column, and determined by spectrophotometer (Keeney and Nelson 1982). In total, thirteen soil chemistry properties were assayed (Table 3).

Statistical Analysis of Soil Chemistry

Soil chemistry data for the 52 sampled *Ceanothus* populations were treated using univariate and multivariate statistical methods. First, differences among pre-defined groups were tested for each of the 13 soil chemistry variables using Student's paired t-tests (Student 1908). Second, differences among groups were summarized using principal component analysis (PCA; Pearson 1901), which simultaneously accounted for variation in all 13 soil chemistry variables. Principal component analysis was carried out in the program R, version 2.10.1 (R Development Core Team 2009), using the "ecodist" package of Goslee and Urban (2007). The soil chemistry variables were transformed into Z-scores prior to analysis. The first two principal components were visualized in bivariate space and the relative contribution of the soil chemistry variables to the components was assessed based on vector loadings. Based on PCA scores, differences among pre-defined groups were tested using a combination of 1) Student's paired t-test, 2) analysis of variance (ANOVA; Fisher 1918), and 3) Tukey's HSD test (Zar 1999). Comparisons involving just two groups were carried out using Student's paired t-test (Student 1908) on scores from the first two principal components. To test for overall differences among three or more groups, one-way ANOVA was carried out on scores from the first two principal components. For analyses yielding a significant ANOVA result, Tukey's HSD (Honestly Significant Difference) test was used to determine which groups were significantly different from one another. Tukey's HSD test

TABLE 3. SUMMARY STATISTICS FOR SOIL CHEMISTRY VARIABLES. All values given as group mean ± standard deviation. Analysis group = groups of soil samples treated in text; "*C. cuneatus* all samples" refers to all soil samples analyzed for *C. cuneatus* (Table 2); "*C. cuneatus* El Dorado non-gabbro" and "*C. cuneatus* El Dorado gabbro" refer to *C. cuneatus* populations collected in El Dorado Co., CA on non-gabbro and gabbro-derived soils, respectively. *C. roderickii*, n = 10; *C. cuneatus* all samples, n = 42; *C. cuneatus* El Dorado non-gabbro, n = 9; *C. cuneatus* El Dorado gabbro, n = 6. Con. = electrical conductivity of soil, reported as umho/cm; nitrate and all other nutrient levels reported as PPM.

Analysis group	pH	Con.	Nitrate	Mg	Ca	K	P	S	Na	Fe	Zn	Mn	Cu
C. roderickii	6.0 ± 0.1	78 ± 16	5.0 ± 1.8	633 ± 179	1744 ± 208	60.7 ± 11.9	2.2 ± 0.8	9.6 ± 1.2	65.8 ± 6.8	7.0 ± 1.8	0.4 ± 0.3	13.1 ± 3.3	1.6 ± 0.8
C. cuneatus all samples	6.1 ± 0.5	83 ± 53	8.5 ± 7.5	573 ± 573	1513 ± 862	129.3 ± 7.5	19.2 ± 21.2	10.9 ± 3.0	84.7 ± 36.4	16.6 ± 10.7	1.1 ± 1.3	14.9 ± 10.8	1.0 ± 1.6
C. cuneatus El Dorado non-gabbro	6.2 ± 0.4	75 ± 16	6.5 ± 3.9	972 ± 564	1355 ± 653	94.5 ± 3.9	8.5 ± 8.9	9.1 ± 1.7	63.1 ± 5.0	17.2 ± 13.2	0.8 ± 0.4	16.0 ± 15.4	0.7 ± 0.3
C. cuneatus El Dorado gabbro	6.1 ± 0.3	97 ± 20	8.4 ± 5.0	474 ± 132	2450 ± 333	117.0 ± 5.0	15.8 ± 21.1	13.1 ± 1.4	71.8 ± 9.8	12.5 ± 4.5	2.3 ± 2.5	26.9 ± 8.9	4.2 ± 2.3

compensates for false positives (type I error) in multiple comparisons and therefore reveals which differences among group means are "honestly" significant (Zar 1999). Student's paired t-tests, One-way ANOVA, and Tukey's HSD tests were carried out in R (R Development Core Team 2009). In all statistical tests the threshold of significance was $\alpha = 0.05$.

RESULTS

DNA Sequences

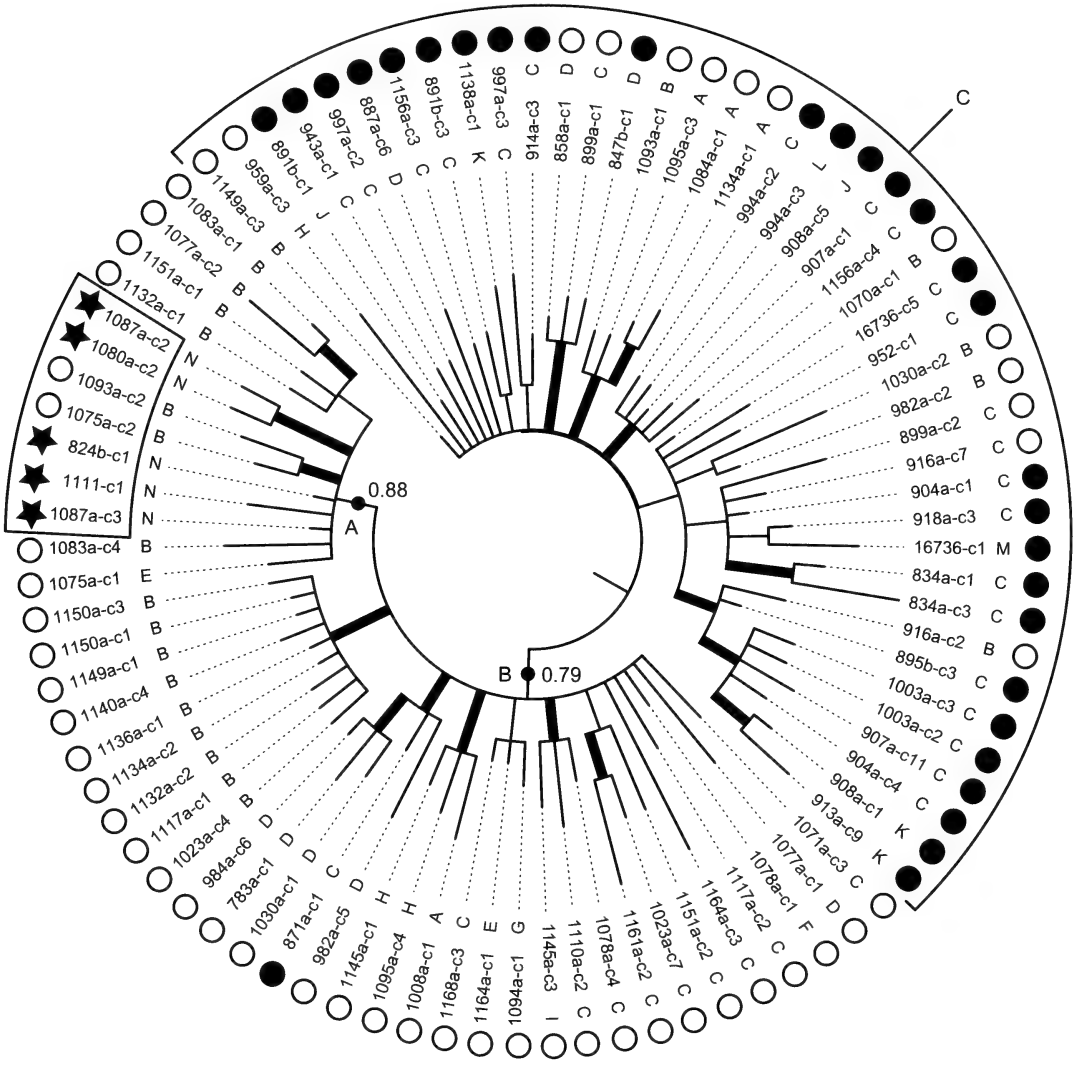
The portion of the third intron of nitrate reductase selected for analysis varied in length from 558 bp (*C. cuneatus* var. *cuneatus* isolates DOB 1136a-c1, 1140a-c4, 1149a-c1, 1023a-c4, 1117a-c1, 1134a-c2, and 1150a-c1; Table 2, Appendix 1) to 670 bp (*C. cuneatus* var. *cuneatus* isolates DOB 1084a-c1 and 1134a-c1). The initial alignment of the 87 NIA isolates selected for analysis (Table 2, Appendix 1) contained 694 characters and had an average G + C content of 37.1% (TreeBase Study S10898, Matrix M6862). Following exclusion of two ambiguously aligned regions (base positions 136–152 and 401–448; see Materials and Methods), the alignment contained 618 characters, 149 of which (24.1%) were variable. The ambiguously-aligned regions were excluded from all subsequent analyses.

Phylogeny

Bayesian analysis replicates had a burn-in period of 500,000 generations (500 sampled trees), leaving 4,500,000 generations (4500 sampled trees) of explored tree space for computing branch lengths and posterior probabilities (PP) of clades. The three Bayesian replicates yielded trees with nearly identical topology. Only one run was used for final tree building.

In the Bayesian consensus tree neither *C. roderickii* nor *C. cuneatus* var. *cuneatus* are recovered as monophyletic (Fig. 3). Instead, *C. roderickii* and *Ceanothus cuneatus* var. *cuneatus* are polyphyletic. All five NIA isolates from the four *C. roderickii* individuals included in this study (Table 2, Appendix 1) are recovered as members of a clade of NIA isolates that are otherwise from individuals of *C. cuneatus* var. *cuneatus* (Fig. 3, Clade A; PP 0.88). Clade A is in turn nested within a larger clade made up almost entirely of isolates from individuals of *C. cuneatus* var. *cuneatus* (Fig. 3, Clade B; PP 0.79). Considering *C. cuneatus* var. *cuneatus* as a whole, seven isolates are recovered in strongly supported (PP > 0.95) relationships with isolates from other taxa, including other varieties of *C. cuneatus* and other species of *Cerastes* (Fig. 3).

Out of the 30 *Ceanothus* plants from which two NIA sequence types were recovered (Table 2,



Type	Variable region motif
A	TTTTAAACAAAA-TTA
B	TTTTTAAAAAAA-TTA
C	TTTTAAAAAAAA-TTA
D	TTTAAAAAAAAA-TTA
E	TTTTTAAAAAAAA-TA
F	TTTTAAAAAGAA-TTA
G	TTTTTTAAAAAAA-TA
H	TTTTAAAAAAA--TTA
I	TTTTAAAATAAAATTA
J	TTTTAACAAAAA-TTA
K	TTTTTAAAAAAAATTA
L	TTTTAAAAAAA--TTG
M	TTTTTAAAAGAAATTA
N	TTTTTTAAA-AAATTA

Figure key

C. cuneatus var. *cuneatus*

C. roderickii

Other *Cerastes*

0.09 Substitutions/site

1101-c1 A Isolate code and motif type

FIG. 3. Bayesian 50% majority-rule consensus phylogram for nitrate reductase. Heavy bars indicate posterior probability >0.95. Phylogram is manually rooted based on root position inferred from expanded *Ceanothus* phylogeny (see Materials and Methods). Highly-variable region motifs from NIA (see Materials and Methods) shown below phylogram; motif types mapped on phylogram using letter codes. "A, B, C": groups and clades discussed in text; numbers on branches indicate posterior probabilities. All NIA isolates from DOB collections, with exception of 16736-c1 from D.H. Wilken 16736 (Table 2; Appendix 1).

Appendix 1), 21 have these isolates in conflicting positions on the phylogeny (Fig. 3; PP > 0.95). Of the remaining nine plants from which two NIA sequence types were recovered, five have both isolates as members of a single well-supported clade (PP > 0.95), and four have isolates that are neither strongly supported as members of the same clade, nor in conflicting phylogenetic positions (Fig. 3).

Gene Genealogy

Among the 87 NIA isolates included in the analysis, TCS identified 82 unique sequences. Three of these are represented by more than one NIA isolate (Fig. 4), one comprising four isolates from individuals of *C. cuneatus* var. *cuneatus* collected in the southern Sierra Nevada (1150a-c1, 1149a-c1, 1136a-c1, and 1134a-c2), a second comprising two isolates from individuals of *C. cuneatus* var. *cuneatus* collected in the northern Sierra Nevada of California and Cascade Ranges of Oregon (1164a-c1 and 1168a-c3), and a third represented by two isolates from individuals of *C. roderickii* collected in different populations (1087a-c3 and 824b-c1). All remaining sequence types are unique. The gene genealogy inferred by TCS is reticulate, with 22 loops (ambiguities) as reconstructed (Fig. 4).

Highly-Variable Region Motifs

Among the 87 NIA isolates utilized in the study, a total of 14 motif types were identified for the first highly-variable region (Materials and Methods; Fig. 3). The "N" motif (Fig. 3) is unique to *C. roderickii* and was present in all 16 isolates (four per individual plant) obtained from individuals of this species, as well as 16 isolates obtained from 4 additional individuals of the species collected in different populations or subpopulations (unpublished data). Nine motif types are found in *Ceanothus cuneatus* var. *cuneatus* (Fig. 3). Seven of these types are unique to *C. cuneatus* var. *cuneatus*, including three known from just a single NIA isolate each (F, G, and I). The remaining two motifs recovered in *C. cuneatus* var. *cuneatus* (C and D) are shared with other varieties of *C. cuneatus* or other *Cerastes* species (Fig. 3). None of the motifs from *C. cuneatus* var. *cuneatus* is unique to a well-supported group of *C. cuneatus* var. *cuneatus* isolates in the NIA tree (Fig. 3), although the "B" motif is found predominantly in Clade B (Fig. 3; PP 0.79) and is found in all but one of the *C. cuneatus* var. *cuneatus* isolates that group with *C. roderickii* in Clade A (Fig. 3; PP 0.88). Four additional motifs (J, K, L, and M; Fig. 3) are found only in taxa other than *C. cuneatus* var. *cuneatus* and *C. roderickii*. Two of these are found in more than one taxon (J and K; Fig. 3), and two are unique to a particular isolate (L and M; Fig. 3).

Soil Analyses

At a large geographic scale (Fig. 2), considering all 52 soil samples collected within populations of *C. cuneatus* (all five varieties) and *C. roderickii* (Tables 1 and 2), the soils of *C. roderickii* have, on average, lower pH, lower electrical conductivity, and lower concentrations of nitrate, K, P, S, Na, Fe, Zn, and Mn (Table 3, *C. roderickii* vs. *C. cuneatus* all samples). Concentrations of Mg, Ca, and Cu, on the other hand, are on average higher in the soils of *C. roderickii* than in those of *C. cuneatus* (Table 3). Differences are significant in the case of K, P, S, Fe, and Zn (Student's paired t-tests, $P < 0.03$). Principal component analysis summarizes these results for the 13 soil chemistry variables. In PCA the first two principal components account for 39% of total variance, with 21% on the first principal component and 19% on the second. The first principal component is strongly positively correlated with Mg (vector loading = 0.48) and electrical conductivity (vector loading = 0.34), and strongly negatively correlated with P (vector loading = 0.46) and K (vector loading = 0.41). These results are summarized in a biplot of the first two principal components (Fig. 5A). Student's paired t-tests allow for rejection of the null hypothesis of no difference between the mean PCA scores for *C. roderickii* and *C. cuneatus* on the second principal component ($P < 0.001$; Fig. 5B) but not the first ($P = 0.052$).

At a smaller geographic scale (Fig. 1), considering only those 28 soil samples collected in El Dorado Co., California (Tables 1 and 2), there are differences in chemistry between soils of *C. roderickii* and *C. cuneatus* var. *cuneatus* that are partitioned with respect to both taxon and geological parent material (Table 3). Within this geographic region *C. cuneatus* var. *cuneatus* grows on soils derived from a variety of geological parent materials, including gabbro (Tables 1 and 2). In comparison to *C. roderickii*, soils of *C. cuneatus* var. *cuneatus* that are derived from non-gabbro parent material (including serpentinite; Table 2; see below) have, on average, higher pH and higher concentrations of nitrate, Mg, K, P, Fe, Zn, and Mn (Table 3, *C. roderickii* vs. C. *cuneatus* El Dorado non-gabbro). Electrical conductivity and concentrations of Ca, S, Na, and Cu, on the other hand, are lower in soils of *C. cuneatus* var. *cuneatus* derived from non-gabbro parent material than in the soils of *C. roderickii* (Table 3). Differences are significant in the case of Fe, Zn, and Cu (Student's paired t-tests, $P < 0.04$). Comparing the exclusively gabbro-derived soils of *C. roderickii* to the soils of *C. cuneatus* var. *cuneatus* that are also derived

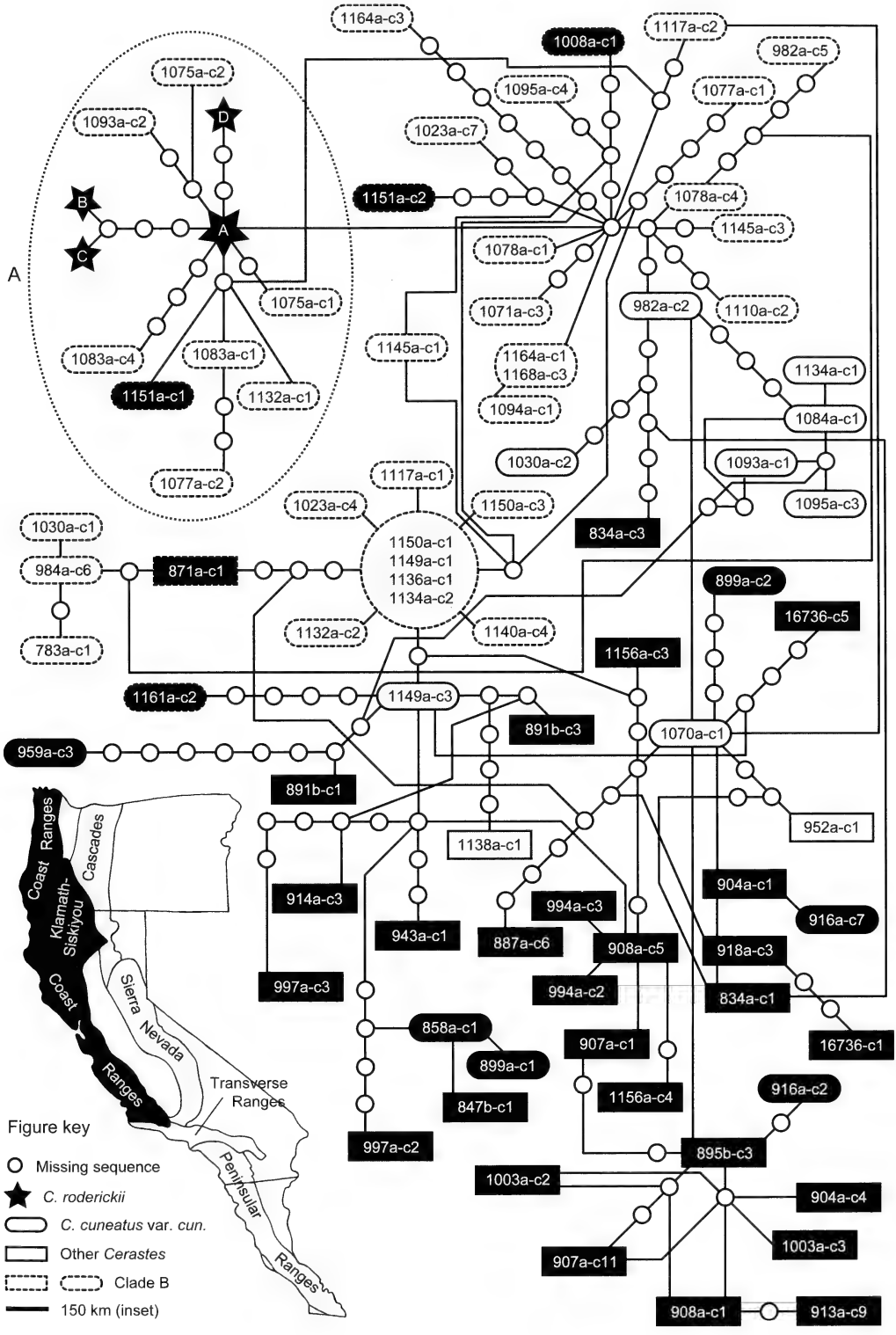


FIG. 4. Gene genealogy of NIA isolates generated under the statistical parsimony criterion in the program TCS (Clement et al. 2000). Open circles represent un-sampled (missing) sequences, as inferred by TCS. Some branch lengths not proportional to number of substitutions. *Ceanothus roderickii* (solid black stars): A, 1087a-c3 & 824b-c1; B, 1080a-c2; C, 1087a-c2; D, 1111-c1. "Clade A": group discussed in text. Sequences color-coded according to geography (see inset map).

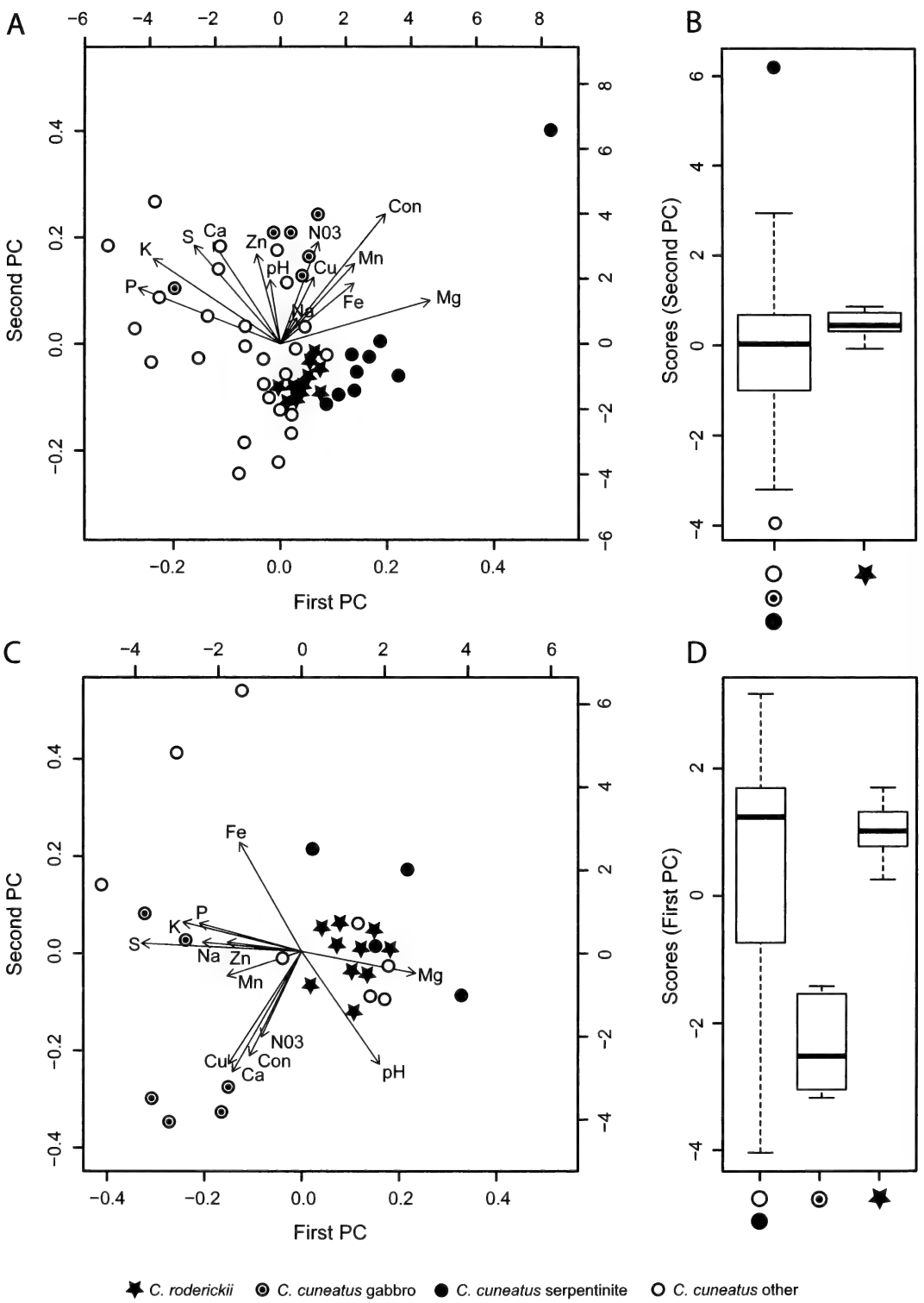


FIG. 5. Plots from principal component analysis (PCA) of soil chemistry data. A, biplot for first two principal components of PCA on soil chemistry for 52 assayed soil samples; arrows represent direction and magnitude of loading on the principal component axes; bottom and left axes apply to loading; top and right axes apply to PCA scores. B, boxplot of PCA scores from the second principal component of PCA on all 52 assayed soil samples, partitioned by species. C, biplot for first two principal components of PCA on soil chemistry for all soils collected in El Dorado County, CA; axes as in A. D, boxplot of PCA scores from the first principal component of PCA on El

from gabbro parent material, the gabbro-derived soils of *C. cuneatus* var. *cuneatus* have, on average, higher pH, higher electrical conductivity, and higher concentrations of nitrate, Ca, K, P, S, Na, Fe, Zn, Mn, and Cu (Table 3). Mg is the only element that is present in lower concentrations in soils of *C. roderickii* than in gabbro-derived soils of *C. cuneatus* var. *cuneatus*. Differences are significant for K, Ca, F, S, Fe, Mn, and Cu (Student's paired t-tests, $P < 0.04$). Principal component analysis summarizes these results for the 13 soil chemistry variables. In PCA the first two principal components account for 45% of total variance, with 27% on the first principal component and 18% on the second. The first principal component is strongly positively correlated with Mg (vector loading = 0.35) and pH (vector loading = 0.24), and strongly negatively correlated with S (vector loading = 0.49) and K (vector loading = 0.36). These results are summarized in a biplot of the first two principal components (Fig. 5C). Differences among the exclusively gabbro-derived soils of *C. roderickii*, the gabbro-derived soils of *C. cuneatus* var. *cuneatus*, and the non-gabbro derived soils of *C. cuneatus* var. *cuneatus* were tested using ANOVA on PCA scores (Fig. 5C). ANOVA allowed for rejection of the null hypothesis of no difference among the three group means on the basis of the first principal component ($F = 10.96$; $P < 0.001$; Fig. 5D) as well as the second ($F = 6.34$; $P = 0.006$). Tukey's HSD test allowed for rejection of the null hypothesis of no difference between the gabbro-derived and non-gabbro derived soils of *C. cuneatus* var. *cuneatus* ($P = 0.002$), as well as between the gabbro-derived soils of *C. cuneatus* var. *cuneatus* and those of *C. roderickii* ($P < 0.001$). The mean PCA scores for the non-gabbro derived soils of *C. cuneatus* var. *cuneatus* was not significantly different from those of *C. roderickii* ($P = 0.611$).

Comparing the gabbro-derived soils of *C. roderickii* and *C. cuneatus* var. *cuneatus* to serpentinite-derived soils of *C. cuneatus* (including *C. cuneatus* var. *cuneatus* and *C. cuneatus* Nutt. var. *ramulosus* Greene), there are strong differences among groups. Average Ca:Mg for serpentinite-derived soils of *C. cuneatus* ($n = 8$; Table 2) was 0.6 (standard deviation = 0.3), the average for soils of *C. roderickii* ($n = 10$) was 2.9 (± 0.6), the average for the gabbro-derived soils of *C. cuneatus* var. *cuneatus* ($n = 6$) was 5.5 (± 1.5), and the average for all "other" (non-gabbro and non-serpentinite derived) soils occupied by *C. cuneatus* ($n = 27$) was 7.2 (± 4.1). The difference in Ca:Mg is significant for all three contrasts among 1) the exclusively gabbro-derived soils of *C. roderickii,* 2) the gabbro-derived soils of *C. cuneatus* var. *cuneatus* and 3) the serpentinite-derived soils of *C. cuneatus* (Student's paired t-tests, $P < 0.01$). Overall differences in soil chemistry among these groups are summarized in a biplot of the first two principal components from the PCA described above (Fig. 5A). The differences in soil chemistry among the three groups listed above, as well as the "other" group (non-gabbro and non-serpentinite derived soils occupied by *C. cuneatus*) were tested using ANOVA in terms of scores on the second principal component of the PCA (Fig. 5A). ANOVA allowed for rejection of the null hypothesis of no difference among group means ($F = 5.01$; $P = 0.004$). Furthermore, Tukey's HSD test allowed for rejection of the null hypothesis of no difference between means for two contrasts among the four groups listed above: a) gabbro-derived soils of *C. cuneatus* var. *cuneatus* versus "other" (non-gabbro & non-serpentinite derived) soils of *C. cuneatus* ($P = 0.014$), and b) gabbro-derived soils of *C. cuneatus* var. *cuneatus* versus those of *C. roderickii* ($P = 0.002$). The remaining three contrasts among the four groups were not significant.

Discussion

Phylogenetic Relationships

Our results indicate a very close relationship between the gabbro-endemic *C. roderickii* and the less soil-specialized *C. cuneatus* var. *cuneatus*. Nevertheless, relationships among the 87 NIA isolates included in this study are poorly resolved, with few nodes receiving high levels of support (Fig. 3). This result is consistent with past genetic work on *Cerastes* as a whole, in which nuclear and chloroplast DNA sequence data failed to resolve species-level relationships (Hardig et al. 2000; 2002). Nevertheless, a lack of phylogenetic signal is consistent with the hypothesis that *Cerastes* diversified recently, perhaps as late as 5 mya (Ackerly et al. 2006; Burge et al. in press). If the diversification of *Cerastes* took place during so short a time interval, then a lack of phylogenetic resolution is not unexpected. In addition, genetic divergence among taxa might be further eroded by hybridization, which is common among *Cerastes* taxa and has long been

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Dorado County soil samples, partitioned by species-soil group (see Results). "*C. cuneatus* gabbro" corresponds to soil samples obtained from *C. cuneatus* populations growing on soils derived from gabbro parent material; "*C. cuneatus* serpentinite" corresponds to serpentinite parent material, and "*C. cuneatus* other" to non-gabbro and non-serpentinite parent materials. Symbols: Con = electrical conductivity; N03 = nitrate.

thought to play an important role in *Cerastes* evolution (McMinn 1942; Nobs 1963).

In spite of the low phylogenetic resolution achieved in the present study using NIA, comparison of phylogenetic results with the gene genealogy and information from highly-variable region motifs (Figs. 3, 4) allows for interpretation of the relationship of *C. roderickii* to remaining *Cerastes*. All of the NIA isolates obtained from *C. roderickii*, representing four populations, are nested within a small clade made up of NIA isolates from *C. cuneatus* var. *cuneatus* populations sampled in the Sierra Nevada and Cascade mountains of California (Fig. 3, Clade A). This group is also present in the gene genealogy for NIA, in which only two potential connections were reconstructed between this group and remaining isolates (Fig. 4 "Clade A"). The close relationship between *C. roderickii* and *C. cuneatus* var. *cuneatus* is further emphasized by the nested position of Clade A within Clade B, which is made up almost entirely of isolates from *cuneatus* var. *cuneatus* (Fig. 3). However, it is important to note that members of Clade B are more strongly connected to the remaining NIA isolates in the gene genealogy than are members of Clade A (Fig. 4). Finally, all NIA isolates from *C. roderickii* contained an identical highly-variable region motif that has proven unique to *C. roderickii* (Fig. 3, type N). The type N motif was present in all 20 isolates obtained from *C. roderickii*. Four *C. roderickii* individuals representing additional populations were also found to share the type N highly-variable region motif (unpublished data). The presence of a unique highly-variable region motif in all sampled *C. roderickii* individuals indicates that *C. roderickii* populations are genetically cohesive, in spite of the fact that they do not form a clade in the phylogeny reconstructed using complete NIA sequences (Fig. 3). Thus, the type N highly-variable region motif may be taken as a genetic autapomorphy of *C. roderickii*.

Genetic evidence for the cohesiveness of *C. roderickii* with respect to *C. cuneatus* is supported by the morphology of *C. roderickii*, which differs from that of *C. cuneatus* in several significant ways. First, the habit of *C. roderickii* is always prostrate to decumbent, with shrubs rarely attaining more than a meter in height (Knight 1968; James 1996), whereas *C. cuneatus* is invariably erect and ascending, frequently reaching more than three meters is height (Fross and Wilken 2006). However, some populations of *C. cuneatus* in the Sierra Nevada and Klamath-Siskiyou region of California and Oregon are much lower-growing (Fross and Wilken 2006). *Ceanothus roderickii* also differs from *C. cuneatus* with respect to mode of reproduction. Individuals of *C. roderickii* spread laterally via arching or creeping branches that root adventitiously when they contact soil. This mode of reproduction allows *C. roderickii* to reproduce clonally during fire-free intervals when seedling recruitment is limited (James 1996; Boyd 2007). Clonal reproduction is not known in *C. cuneatus*. Finally, the leaves of *C. roderickii* are usually strongly ascending (Knight 1968), such that the leaf surface is typically held perpendicular to the soil surface. Few other *Cerastes* species are known to possess this trait (Knight 1968), which may represent an adaptation to the very high light levels that are typical of the open habitats favored by *C. roderickii* (James 1996).

Overall, phylogenetic findings of the present study agree with previous systematic work on *C. roderickii*. Citing general similarities in habit, ecology, and geographic distribution, Knight (1968) argued that *C. roderickii* is probably most closely related to *C. cuneatus*, although he did not rule-out the possibility of a relationship with several other *Cerastes* species from the Sierra Nevada. The results of the present study also agree with those of Hardig et al. (2000), in which an individual of *C. roderickii* grouped with an individual of *C. cuneatus* var. *cuneatus* in phylogenies based on sequences from ITS and *matK*.

In addition to the relationship between *C. roderickii* and *C. cuneatus*, results presented here bear on relationships among other *Cerastes* included in the taxonomically diverse clade that is the focus of the present study (Table 1). The Bayesian consensus tree contains a moderately supported clade comprising 38 out of the 52 NIA isolates from *C. cuneatus* var. *cuneatus,* all of the isolates for *C. roderickii*, and a single isolate from *Ceanothus cuneatus* Nutt. var. *fascicularis* (McMinn) Hoover (Fig. 3, Clade B, PP 0.79). Clade B is made up almost entirely of NIA isolates from *C. cuneatus* var. *cuneatus* individuals collected in the mountains of Baja California, Mexico, southern California, eastern California, and eastern Oregon, which includes the Sierra Nevada, Cascade Ranges, Peninsular Ranges, and Transverse Ranges (Fig. 4). Although the relationship is less obvious than in the Bayesian consensus tree (Fig. 3), Clade B and its unusual geography is recognizable in the gene genealogy, which contains few connections between members of Clade B and remaining isolates (Fig. 4). The relationship between Clade B and remaining NIA isolates is not resolved in the Bayesian consensus tree (Fig. 3); several small clades of isolates, as well as some individual isolates, form a large polytomy with Clade B (Fig. 3, Group C). In an expanded analysis of *Ceanothus* phylogeny (Burge et al. in press) the root of our tree (Fig. 3) falls within this polytomy, indicating that a lack of resolution here is not an artifact of sampling.

All but 10 of the plants represented by the Group C isolates were collected in the Klamath-Siskiyou and Coast Ranges of California, the

exceptions being seven isolates from individuals of *C. cuneatus* var. *cuneatus* collected in the Sierra Nevada, Peninsular Ranges, and Transverse Ranges (1149a-c3, 1095a-c3, 1084a-c1, 1134a-c1, 1070a-c1, 1030a-2, and 982a-c2), one isolate of *C. cuneatus* var. *cuneatus* collected in the Sutter Buttes (1093a-c1), and one isolate each from individuals of *C. fresnensis* (1138a-c1) and *C. prostratus* (952a-c1) collected in the Sierra Nevada (Fig. 4).

The genetic break between the Klamath-Siskiyou/Coast Ranges and the remaining CFP mountains (Sierra Nevada, Peninsular Ranges, and Transverse Ranges; Fig. 4) appears to represent a biogeographic split between *Cerastes* inhabiting these regions, although the presence of isolates from individuals collected in the Klamath-Siskiyou/Coast Ranges within Clade B, and the presence of individuals collected in other mountain ranges of the CFP in Group C, suggests that opportunities for migration and/or gene-flow between the regions have been available (Figs. 3, 4). In addition, the frequent lack of monophyly between NIA isolates from the same individual (Fig. 3), including 6 cases in which isolates from a single individual are found in both Clade B and Group C (1093a, 1149a, 1134a, 1030a, 982a, 1095a), suggests the operation of gene-flow or hybridization. Hybridization and gene flow are thought to be common in *Cerastes* (McMinn 1942; Nobs 1963; Fross and Wilken 2006), and so it is not unexpected to find evidence consistent with these phenomena.

Edaphic Ecology

At a large geographic scale, considering all sampled populations of *C. cuneatus* and *C. roderickii* (Fig. 2), results of our study show that edaphic conditions experienced by the narrowly distributed gabbro-endemic *C. roderickii* represent a small, highly cohesive subset of the range of conditions experienced by the widespread soil-generalist *C. cuneatus* (Fig. 5A, B). Soils of *C. roderickii* are characterized by low concentrations of available K, P, S, Fe, and Zn, all of which are necessary plant nutrients (Brady and Weil 2002). For many plants, low availability of these elements results in disorders affecting growth and reproduction (Brady and Weil 2002).

At the scale of the Pine Hill intrusive complex in western El Dorado Co., California (Fig. 1), our study shows that *C. roderickii* is specialized to nutrient-deficient forms of gabbro-derived soil (Fig. 5C, D). On the Pine Hill intrusive complex *C. cuneatus* var. *cuneatus* and *C. roderickii* both occur on soils that are considered gabbro-derived (Fig. 1). However, the gabbro-derived soils of *C. roderickii* sampled in our study, which are classified as "Rescue extremely stony sandy loam" (Rogers 1974), contain significantly lower levels of K, Ca, P, S, Fe, Mn, and Cu than gabbro-derived soils of *C. cuneatus* var. *cuneatus* ($P < 0.04$; Table 3), which are classified as "Rescue sandy loam" or "Rescue very stony sandy loam" (Fig. 1; Rogers 1974). Although these elements are necessary plant nutrients, high levels of some, such as Mn, Fe, and Cu, are known to induce growth and reproductive disorders in plants (Brady and Weil 2002). Our work is the first to report this strong soil-chemistry divergence between *C. cuneatus* var. *cuneatus* and *C. roderickii* on the Pine Hill intrusive complex.

The relatively higher fertility of gabbro-derived soils occupied by *C. cuneatus* var. *cuneatus* compared to those occupied by *C. roderickii* may result from the greater development of the former, which are typically found in swales and at the bases of steep slopes, where they receive run-off from the Rescue extremely stony sandy loams that are found on the steeper slopes, hills, and ridge crests of the Pine Hill intrusive complex (Rogers 1974; D.O. Burge, personal observation). While our study is the first to report significantly divergent chemistry between groups of gabbro-derived soils on the Pine Hill intrusive complex, similar phenomena are known from other soils; on some serpentinite outcrops, soils at the base of steep slopes have strongly divergent chemistry from the soils closer to the top of the slope, despite their common geological parent material (Rajakaruna and Bohm 1999).

Endemism on gabbro-derived soils of the Pine Hill intrusive complex, as well as the presence on these soils of many taxa normally restricted to serpentinite-derived substrates, have been attributed to similar properties in gabbro-derived as compared to serpentinite-derived soils (Wilson 1986). Soils derived from serpentinite contain little Ca relative to Mg, and are rich in heavy metals such as Ni, Cr, and Co (Kruckeberg 2002). Gabbro rock itself is usually rich in heavy metals and tends to contain little Ca relative to Mg, although these parameters are not as extreme in gabbro as in serpentinite (Alexander 1993, unpublished). Research on the Pine Hill intrusive complex, however, found that the gabbro-derived soils from this area do not contain unusually low levels of Ca relative to Mg, or elevated heavy metals (Hunter and Horenstein 1991), results that are corroborated by regional geochemical studies (Goldhaber et al. 2009; Morrison et al. 2009). A later study focused on the gabbro-endemic plants of the Pine Hill intrusive complex asked whether soils from locations harboring endemics had low Ca to Mg ratios, or differences in a suite of other chemical and physical parameters, compared to areas without these plants (Alexander, unpublished). This study did not detect significant differences in Ca to Mg ratio between sites harboring rare plants versus those without, and

failed to identify other parameters that might explain the differences in plant distribution. However, it is possible that the results of this study were confounded by plant demography. This may be especially true of *C. roderickii*, which depends on fire for recruitment (Boyd 2007).

Although the present study did not focus on the contrast between serpentinite and gabbro, our results show that Ca to Mg ratios in serpentinite-derived soils of *C. cuneatus* (average 0.6 ± 0.3) are closest to those in the exclusively gabbro-derived soils of *C. roderickii* (average 2.9 ± 0.6). Values become successively higher in gabbro-derived soils of *C. cuneatus* var. *cuneatus* (5.5 ± 1.5), and "other" (non-gabbro and non-serpentinite derived) soils of *C. cuneatus* (7.2 ± 4.1). Although soils of *C. roderickii* have Ca to Mg ratios that are closest to those in serpentinite-derived soils, ratios in serpentinite-derived soils are still significantly lower (Student's paired t-test, $P < 0.001$). Nevertheless, serpentinite-derived soils associate closely with the exclusively gabbro-derived soils of *C. roderickii* in PCA (Fig. 5A, C). Furthermore, the two groups are not significantly different in terms of their scores on these axes (Tukey's HSD test, $P = 0.489$), indicating that the serpentinite-derived soils are similarly nutrient deficient. Overall, nutrient deficiency and low Ca to Mg ratios may provide an explanation for the evolution of endemics on some gabbro-derived soils of the Pine Hill intrusive complex, and the presence on these soils of plants that are usually restricted to serpentinite-derived substrates (Wilson 1986).

Evolution of Edaphic Ecology

Evolution of the gabbro-endemic *C. roderickii* appears to have been associated with specialization to strongly nutrient-deficient forms of gabbro-derived soil. The closest relative of *C. roderickii*, *C. cuneatus* var. *cuneatus*, has a very wide distribution in the California Floristic Province (Fig. 2), and is a common component of chaparral habitats in the Sierra Nevada. On the Pine Hill intrusive complex of western El Dorado Co., California, *C. cuneatus* var. *cuneatus* occupies nutrient-rich forms of gabbro-derived soils in close geographic proximity to the poorer forms favored by *C. roderickii*, sometimes no more than 100 m distant from the latter species (Fig. 1).

Although there is not a well-supported "progenitor-derivative" relationship (Gottlieb 2003; Baldwin 2005) between *C. cuneatus* var. *cuneatus* and *C. roderickii*, the nested position of *C. roderickii* within a large group of *C. cuneatus* var. *cuneatus* individuals collected predominantly in the Sierra Nevada, Transverse Ranges, and Peninsular Ranges is suggestive of this pattern (Figs. 3, 4). Rocks of the Pine Hill intrusive complex have probably been exposed since Eocene time (J. Wakabayashi, personal communication). Thus, it is possible that during the diversification of *Ceanothus* in western North America, which began approximately 5 mya (Ackerly et al. 2006; Burge et al. in press), *C. cuneatus* var. *cuneatus* colonized the Pine Hill region and gave rise to *C. roderickii* through specialization to the nutrient-poor forms of gabbro-derived soil.

Because intrinsic (pre-zygotic) barriers to gene flow are not known in *Cerastes* (Nobs 1963), it is expected that hybridization will occur when different species come into contact with one another (Fross and Wilken 2006), potentially leading to gene flow and introgression. However, *C. roderickii* persists as a relatively genetically isolated, morphologically divergent entity in spite of its close proximity to *C. cuneatus* var. *cuneatus* on the Pine Hill intrusive complex (Fig. 1). One possible explanation for the lack of introgression is the action of environmental isolating factors. The fact that soil chemistry associations of *C. cuneatus* var. *cuneatus* and *C. roderickii* are most divergent where the taxa come into close contact on gabbro outcrops, with *C. cuneatus* var. *cuneatus* occupying comparatively nutrient-rich forms of gabbro-derived soil, is suggestive of character displacement and possibly reinforcement based on soil-chemistry (Levin 1970). Overall, edaphically-based barriers to gene-flow might provide an explanation for the initial divergence and continued persistence of *C. roderickii*, as well as other edaphic-endemic *Cerastes* taxa.

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APPENDIX 1

SAMPLED *CEANOTHUS* POPULATIONS

GenBank accession numbers for the first and second NIA sequence (where available) are in brackets []. See Table 2 for additional population information.

Ceanothus cuneatus* Nutt. var. *cuneatus—USA. CALIFORNIA. **Amador Co.**: Grass Valley Creek watershed, NE of Mount Zion, *D.O. Burge 1150a* (DUKE) [NIA: HM240330; HM240329]. **Butte Co.**: Feather Falls, *D.O. Burge 1109a* (DUKE); Doe Mill Ridge, *D.O. Burge 815a* (DUKE); Magalia Reservoir, *D.O. Burge 1078a* (DUKE) [NIA: HM240306; HM240307]. **Calaveras Co.**: North Fork Calaveras River watershed, NE of Golden Gate Hill (VABM 2064), *D.O. Burge 1149a* (DUKE) [NIA: HM240327; HM240328]. **Contra Costa Co.**: Mount Diablo State Park, roadside on South Gate Rd, *D.O. Burge 916a* (DUKE) [NIA: HM240341; HM240342]. **El Dorado Co.**: Roadside on Wentworth Springs Rd, 1.9 road mi (3.0 km) from intersection with SR 193, *D.O. Burge 1011a* (DUKE); Green Valley Rd, *D.O. Burge 1024a* (DUKE); Folsom Lake Watershed, W side of North Fork American River arm, S slope of Kelly Ravine, *D.O. Burge 1074a* (DUKE); Martinez Creek watershed, roadside on Pleasant Valley Rd, *D.O. Burge 1174a* (DUKE); Weber Creek watershed, roadside on Lotus Rd, *D.O. Burge 1076a* (DUKE); South Fork American River watershed, *D.O. Burge 1088a* (DUKE); City of Cameron Park, *D.O. Burge 1089a* (DUKE); S side of U.S. Hwy 50, between Durock Rd and U.S. Hwy 50, *D.O. Burge 1101a* (DUKE); Pine Hill, eastern slope, *D.O. Burge 1116a* (DUKE); South Fork American River watershed, Dave Moore Nature Area, *D.O. Burge 1175a* (DUKE); S side of U.S. Hwy 50, between Durock Rd and Hwy 50, *D.O. Burge 1023a* (DUKE) [NIA: HM240297; HM240296]; Tennessee Creek watershed, roadside on Shingle Springs Rd, *D.O. Burge 1075a* (DUKE) [NIA: HM240303; HM240302]; Shingle Creek watershed, S of the city of Cameron Park, *D.O. Burge 1095a* (DUKE) [NIA: HM240314; HM240315]; S shore of Bass Lake, *D.O. Burge 1110a* (DUKE) [NIA: HM240316]; South Fork American River watershed, Icehouse Rd, *D.O. Burge 1117a* (DUKE) [NIA: HM240318; HM240317]. **Fresno Co.**: Dalton Mountain, south-eastern slope, head of Tretten Canyon, *D.O. Burge 1136a* (DUKE) [NIA: HM240323]. **Kern Co.**: Clear Creek watershed, S of Ball Mountain and SE of Hooper Hill, *D.O. Burge 1132a* (DUKE) [NIA: HM240319; HM240320]. **Lake Co.**: Mayacmas Mountains, Cow Mountain Recreation Area, Fourmile Glade, *D.O. Burge 1008a* (DUKE) [NIA: HM240295]. **Los Angeles Co.**: Sierra Pelona Mountians, Ruby Canyon, *D.O. Burge 1071a* (DUKE) [NIA: HM240301]. **Mariposa Co.**: Chowchilla River watershed, East Fork, N of Miami Mountain and E of Paloni Mountain, *D.O. Burge 1140a* (DUKE) [NIA: HM240324]. **Monterey Co.**: Nacimiento-Fergusson Rd, *D.O. Burge 858a* (DUKE) [NIA: HM240338]. **Napa Co.**: Vaca Mountains, on east-west trending ridge S of East Mitchel Canyon, *D.O. Burge 899a* (DUKE) [NIA: HM240339; HM240340]. **Nevada Co.**: Community of Hills Flat, near the City of Grass Valley, *D.O. Burge 1084a* (DUKE) [NIA: HM240310]. **Placer Co.**: North Fork American River Watershed, Forest Hill Divide, *D.O. Burge 1077a* (DUKE) [NIA: HM240305; HM240304]. **Riverside Co.**: San Jacinto Mountains, at intersection of Chimney Flats Rd and USFS Rd 5S13, *D.O. Burge 803a* (DUKE); Tucalota Creek watershed, roadside on Sage Rd (County Rd R3), *D.O. Burge 982a* (DUKE) [NIA: HM240344; HM240345]. **Sacramento Co.**: American River watershed, near outlet of Willow Creek into Lake Natoma, *D.O. Burge 1094a* (DUKE) [NIA: HM240313]. **San Bernardino Co.**: Rialto Municipal Airport (Miro Field), *D.O. Burge 1070a* (DUKE)

[NIA: HM240300]. **San Diego Co.**: Morena Valley, roadside on Buckman Springs Rd, *D.O. Burge 984a* (DUKE) [NIA: HM240346]. **San Luis Obispo Co.**: Santa Lucia Mountains, Arroyo Grande Creek watershed, NW of Arroyo Grande, *D.O. Burge 959a* (DUKE) [NIA: HM240343]. **Shasta Co.**: Crystal Creek watershed, N of Crystal Creek Rd, *D.O. Burge 1151a* (DUKE) [NIA: HM240331; HM240332]. **Sierra Co.**: Goodyears Bar, near confluence of Goodyears Creek and North Yuba River, *D.O. Burge 1083a* (DUKE) [NIA: HM240308; HM240309]. **Sutter Co.**: Sutter Buttes, Peace Valley, *D.O. Burge 1093a* (DUKE) [NIA: HM240312; HM240311]. **Tehama Co.**: Paynes Creek watershed, immediately W of Palmer Gulch, *D.O. Burge 1168a* (DUKE) [NIA: HM240336]. **Tulare Co.**: Middle Fork Tule River, roadside on SR 190, *D.O. Burge 1134a* (DUKE) [NIA: HM240322; HM240321]. **Tuolumne Co.**: Red Hills, SW of Taylor Hill, *D.O. Burge 1145a* (DUKE) [NIA: HM240326; HM240325]. OREGON. **Douglas Co.**: South Umpqua River watershed, roadside on Dole Drive, *D.O. Burge 1161a* (DUKE) [NIA: HM240333]. **Jackson Co.**: Cottonwood Creek watershed, *D.O. Burge 1164a* (DUKE) [NIA: HM240334; HM240335]. MEXICO. **Baja CA**: Sierra San Pedro Martir, Los Llanitos, *D.O. Burge 1030a* (DUKE) [NIA: HM240298; HM240299]; Sierra San Pedro Mártir, 40.4 road mi (64.6 km) E of Mexico Hwy 1, *D.O. Burge 783a* (DUKE) [NIA: HM240337].

***Ceanothus cuneatus* Nutt. var. *dubius* J.T. Howell**—USA. CALIFORNIA. **Santa Cruz Co.**: Henry Cowell Redwoods State Park, *D.O. Burge 918a* (DUKE) [NIA: HM240347].

***Ceanothus cuneatus* Nutt. var. *fascicularis* (McMinn) Hoover**—USA. CALIFORNIA. **Santa Barbara Co.**: Vandenberg Village, *D.O. Burge 871a* (DUKE) [NIA: HM240348].

***Ceanothus cuneatus* Nutt. var. *ramulosus* Greene**—USA. CALIFORNIA. **San Luis Obispo Co.**: Prefumo Canyon, *D.O. Burge 847b* (DUKE) [NIA: HM240349].

***Ceanothus cuneatus* Nutt. var. *rigidus* (Nutt.) Hoover**—USA. CALIFORNIA. **Monterey Co.**: Fort Ord Military Reservation, on hillside W of South Boundary Rd, *D.O. Burge 891b* (DUKE) [NIA: HM240351; HM240350].

***Ceanothus divergens* Parry subsp. *confusus* (J.T. Howell) Abrams**—USA. CALIFORNIA. **Sonoma Co.**: Mayacmas Mountains, western slope of Mount Hood, *D.O. Burge 1003a* (DUKE) [NIA: HM240352; HM240353].

***Ceanothus divergens* Parry subsp. *occidentalis* (McMinn) Abrams**—USA. CALIFORNIA. **Lake Co.**: Boggs Mountain Demonstration State Forest, *D.O. Burge 943a* (DUKE) [NIA: HM240354].

***Ceanothus ferrisiae* McMinn**—USA. CALIFORNIA. **Santa Clara Co.**: Pigeon Point, *D.O. Burge 834a* (DUKE) [NIA: HM240356; HM240355].

***Ceanothus fresnensis* Abrams**—USA. CALIFORNIA. **Fresno Co.**: Big Creek Watershed, E flank of north-south trending ridge W of Ely Mountain, *D.O. Burge 1138a* (DUKE) [NIA: HM240357].

***Ceanothus gloriosus* J.T. Howell var. *exaltatus* J.T. Howell**—USA. CALIFORNIA. **Mendocino Co.**: Oilwell Hill, near the N end of Little Lake Valley, *D.O. Burge 994a* (DUKE) [NIA: HM240358; HM240359].

Ceanothus gloriosus* J.T. Howell var. *gloriosus—USA. CALIFORNIA. **Marin Co.**: Point Reyes National Seashore, *D.O. Burge 908a* (DUKE) [NIA: HM240361; HM240360].

Ceanothus gloriosus J.T. Howell var. *porrectus* J.T. Howell—USA. CALIFORNIA. **Marin Co.**: Point Reyes National Seashore, Inverness Ridge, *D.O. Burge 907a* (DUKE) [NIA: HM240362; HM240363].

***Ceanothus jepsonii* Greene var. *albiflorus* J.T. Howell**—USA. CALIFORNIA. **Colusa Co.**: Rathburn-Petray Mine, *D.O. Burge 997a* (DUKE) [NIA: HM240364; HM240365].

Ceanothus jepsonii Greene var. *jepsonii*—USA. CALIFORNIA. **Marin Co.**: Alpine Lake, *D.O. Burge 914a* (DUKE) [NIA: HM240366].

***Ceanothus maritimus* Hoover**—USA. CALIFORNIA. **San Luis Obispo Co.**: Roadside on Hwy 1, 0.5 road mi (0.8 km) N of bridge over Arroyo de los Chinos, *D.O. Burge 887a* (DUKE) [NIA: HM240367].

***Ceanothus masonii* McMinn**—USA. CALIFORNIA. **Marin Co.**: Golden Gate National Recreation Area, Bolinas Ridge, *D.O. Burge 913a* (DUKE) [NIA: HM240368].

***Ceanothus pinetorum* Coville**—USA. CALIFORNIA. **Trinity Co.**: Un-named rd along ridge, Trinity-Shasta County line, ca. 2.2 linear km SSE of Hoadley Peaks, *D.H. Wilken 16736* (DUKE) [NIA: HM240369; HM240370].

***Ceanothus prostratus* Benth.**—USA. CALIFORNIA. **El Dorado Co.**: El Dorado National Forest, roadside on Wentworth Road, *D.O. Burge 952a* (DUKE) [NIA: HM240371].

Ceanothus pumilus Greene—USA. CALIFORNIA. **Del Norte Co.**: Smith River watershed, near the confluence of Middle Fork Smith River and North Fork Smith River, *D.O. Burge 1156a* (DUKE) [NIA: HM240372; HM240373].

***Ceanothus purpureus* Jeps.**—USA. CALIFORNIA. **Napa Co.**: Wooden Grade, NE of Mount George, *D.O. Burge 904a* (DUKE) [NIA: HM240374; HM240375].

***Ceanothus roderickii* W. Knight**—USA. CALIFORNIA. **El Dorado Co.**: Pine Hill, just E of summit, *D.O. Burge 1080a* (DUKE) [NIA: HM240376]; South Fork American river canyon, near confluence with Weber Creek, *D.O. Burge 1087a* (DUKE) [NIA: HM240377; HM240378]; City of Cameron Park, N side of U.S. Hwy 50, *D.O. Burge 1090a* (DUKE); City of Cameron Park, E of Cameron Airpark, *D.O. Burge 1096a* (DUKE); City of Cameron Park, *D.O. Burge 1100a* (DUKE); S side of U.S. Hwy 50, between Durock Rd and U.S. Hwy 50, *D.O. Burge 1102a* (DUKE); South Fork American River watershed, NW of Mormon Hill, *D.O. Burge 1104a* (DUKE); South Fork American River watershed, NW of Mormon Hill, *D.O. Burge 1105a* (DUKE); Kelley Creek watershed, roadside on Sierrama Rd, *D.O. Burge 1111* (DUKE) [NIA: HM240379]; City of Cameron Park, N side of U.S. Hwy 50, Bureau of Land Management Pine Hill Preserve, *D.O. Burge 1171a* (DUKE); Cameron Park, *D.O. Burge 824b* (DUKE) [NIA: HM240380].

***Ceanothus sonomensis* J.T. Howell**—USA. CALIFORNIA. **Sonoma Co.**: Mayacmas Mountains, head of Hooker Canyon, *D.O. Burge 895b* (DUKE) [NIA: HM240381].

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POLLINATION BIOLOGY OF *DARLINGTONIA CALIFORNICA* (SARRACENIACEAE), THE CALIFORNIA PITCHER PLANT

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ABSTRACT

The pollination ecology of *Darlingtonia californica* Torr., including especially the identity of its pollinators, has remained enigmatic for more than a century. The flowers of this well-known charismatic species are unusual in form and color, and have been the subject of much speculation. Accordingly, in this study we sought to identify *D. californica*'s floral visitors and determine their potential effectiveness as pollinators, in the context of *D. californica*'s unusual floral morphology. We also used hand-pollinations and emasculations to determine whether plants were pollen-limited at five study sites in northwest California, and to evaluate the potential for self-pollination in natural populations of *D. californi*ca. A generalist solitary bee, *Andrena nigrihirta*, visited and pollinated *D. californica* flowers at five sites in northern CA. Despite very low visitation rates, individual flowers at all study sites were predicted to receive at least one visit by *A. nigrihirta*. Other regular floral visitors included thrips and several species of spiders. Plants at all five study populations were found to be pollen-limited with respect to the number of seeds produced per capsule. Fruit and seed production by emasculated flowers indicated a large degree of cross-pollination. However, emasculated flowers did not produce as many fruits and seeds as unmanipulated flowers, suggesting that self-pollination contributes to *D. californica* reproductive success as well. Observations of *A. nigrihirta* on flowers revealed that the shape and orientation of *D. californica*'s ovary and petals promote stigma contact both when pollinators enter and exit a flower, contrary to previous thought. Our findings provide evidence that *D. californica* is melittophilous, and suggest a resolution of the long-standing mystery surrounding the pollination of this rare species.

Key Words: *Andrena nigrihirta*, autogamy, *Darlingtonia californica*, pollination, xenogamy.

The only thing we are lacking is a pollinator.
(Schnell 1976)

The study of the interactions between plants and their pollinators can provide adaptive explanations for floral traits (Harder and Johnson 2009). Careful observation of flower patches —the essential first step in the process—usually generates a list of flower visitors, at least some of which are pollinators. Once the pollinators are known, adaptive hypotheses can be proposed based on an understanding of the biology of the animals as well as the ecological and the phylogenetic context. In spite of the crucial importance of "knowing the pollinators", the pollinator assemblages of a surprising number of plant species remain poorly or entirely unknown. The California pitcher plant, *Darlingtonia californica* Torr., is a case in point. The flowers of this well-known charismatic species are unusual in form and color, and have been the subject of much speculation (Debuhr 1973; Schnell 1976). For example, some have theorized that the bell-shaped ovary serves to limit self-pollination by directing pollinators away from the stigmas as they exit the flowers (Schnell 1976), but very few reports of pollinator visits exist despite serious interest from several workers (Austin 1875–1877 in Juniper et al. 1989; Elder 1997; Nyoka and Ferguson 1999; Nyoka 2000; Rice 2006). Published observations of flower handling by pollinators are lacking. In fact, the pollination ecology of this plant, including especially the identity of its pollinators, has remained enigmatic for more than a century.

The paucity of pollinator sightings is perplexing because fruit set in natural populations of *D. californica* is relatively high, and flowers do not self-pollinate autonomously (Elder 1997; Nyoka 2000). Based on appearances, the flowers of *D. californica* seem adapted for pollination by bees. They are large, showy, sweetly fragrant, and produce abundant pollen (Debuhr 1973; Nyoka and Ferguson 1999)—all features commonly associated with melittophily (Waser 2006). In addition, *D. californica*'s sister taxa, *Sarracenia* and *Heliamphora* spp., are pollinated predominantly by bumble bees (Thomas and Cameron 1986; Renner 1989; Ne'eman et al. 2006), suggesting that bee pollination may be primitive for Sarraceniaceae. Nevertheless, bees have seldom been observed as visitors to *D. californica* flowers (Austin 1875–1877 in Juniper et al. 1989; Elder 1997; Nyoka and Ferguson 1999; Nyoka 2000; Rice 2006). Spiders, in contrast, commonly

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use the flowers as hunting grounds (Austin 1875–1877 in Juniper et al. 1989; Elder 1997; Nyoka 2000). Although arachnids have generally not been given serious consideration as pollinators, Nyoka (2000) noted that they frequently constructed webs and stalked prey inside *D. californica* flowers and carried pollen on their bodies. By experimentally introducing spiders to bagged flowers, she showed that they can cause autogamy. She also found spiders carrying *D. californica* pollen outside of flowers, and detected fluorescent dye particles on spider draglines indicating their potential as cross-pollinators.

The hypothesis that the flowers of *D. californica* are pollinated by spiders is appealing, partly because it would explain why previous workers have seldom seen flying pollinators. However, spider pollination is problematic for at least two reasons. First, the effectiveness of spiders as pollinators may not be sufficient to account for observed levels of fruit and seed set. At her study site in southern Oregon, Nyoka (2000) found that fruit set of open-pollinated flowers approached 100%, and, on average, capsules produced more than 900 seeds. In contrast, fruit set of flowers bagged with spiders was less than 50%, and capsules produced 95% fewer seeds than open-pollinated flowers. This discrepancy implies that other visitors, perhaps bees, play a more important role as pollinators. Second, the morphological fit between spider and flower seems weak at best, making it difficult to conjure plausible adaptive explanations. In fact, the traits that create suitable conditions for spiders, such as the unusually long duration of anthesis (up to 48 days) and the protective tent-like corolla, may be adaptations for bee pollination. Occupation and occasional pollination of *D. californica* flowers by spiders may be incidental and secondary.

In spite of the long history of interest in the pollination of *D. californica*, to date there has been only one published account of a thorough, systematic survey for flower visitors (Nyoka 2000), making it premature to conclude that bees play little or no role in the pollination of the species. Here we report the results of extensive pollinator surveys at five sites in northwestern California. We combine these observations with results of experimental pollination treatments designed to estimate the degree of pollination limitation at these sites as well as the relative importance of self- vs. cross-pollination. The later should provide insight into the relative importance of spiders and bees as pollen vectors, assuming that spiders mainly cause self-pollination. We addressed four specific questions: (1) Who are the most important floral visitors, and are they capable of effecting pollination? (2) Is floral visitation by effective pollinators frequent in natural populations, i.e., is natural pollination sufficient, or are plants experiencing pollen-limitation? (3) Do cross-pollination and self-pollination each contribute to natural pollination? (4) Are past interpretations of the functional morphology of floral traits correct, i.e., does the ovary shape limit self-pollination as has been suggested (Schnell 1976)?

Methods

Study Species

Darlingtonia californica is a carnivorous plant endemic to western Oregon and northern California. Its distribution across this range is patchy, being restricted to perennial wet seeps, generally on serpentine soils (Juniper et al. 1989; Schnell 1976; Whittaker 1954). A long-lived perennial, *D. californica* produces rosettes of leaves from a creeping rhizome every year. Plants often occur in dense patches, which likely result from clonal spread by rhizomes and stolons (Schnell 1976).

The solitary flowers begin as upright buds, but become pendant when mature (Debuhr 1973). Unlike some *Sarracenia* (Ne'eman et al. 2006), the flowers of *D. californica* produce no nectar (Debuhr 1973). Abundant pollen is the only likely reward for pollinators, though a sugar-rich stigmatic exudate may also attract visitors (Nyoka 2000). Five lanceolate-ovate, yellow-green sepals hang loosely around five crimson petals. The five petals almost completely enclose the reproductive whorls, except for windows formed by notches in adjacent petals, which allow access to the flower's interior. The windows are level with the five stigmatic lobes, a feature that has been predicted to promote the deposition of outcrossed pollen as pollinators initially enter a flower (Schnell 1976). Twelve to fifteen stamens are located at the base of the ovary. The bell-shaped ovary is flared towards the stigmas, which has been postulated to function to guide pollinators away from the stigmas as they exit a flower and thus limit self-pollination (Schnell 1976). Flowers mature into upright capsules capable of producing around 2000 seeds (Debuhr 1973). The flowers of *D. californica* are self-compatible, but are not autonomously autogamous (Elder 1997; Nyoka 2000).

Study Sites

Five seeps, located near Scott Mountain and Mt. Eddy, CA were used in this study (Table 1). The five study sites will hereafter be referred to as SM1, SM2, CL, N17, and DF. Distance between sites ranged from ~0.1 to 14.5 km. Near the border of Trinity and Siskiyou counties, this portion of the Klamath Bioregion represents the center of *D. californica's* range (Debuhr 1973). Flowering occurred at all study populations

TABLE 1. ELEVATION AND GEOGRAPHIC COORDINATES OF FIVE STUDY SITES.

Site	Elevation	Spatial coordinates
SM1	1635 m	41°16′25.00″N; 122°41′58.21″W
SM2	1630 m	41°16′38.57″N; 122°41′57.54″W
CL	1693 m	41°18′01.49″N; 122°40′59.90″W
N17	1945 m	41°20′08.05″N; 122°31′41.53″W
DF	2001 m	41°20′09.13″N; 122°31′11.39″W

between June 12, 2008 and June 22, 2008, except for CL where flowering started earlier (June 6, 2008). A total of 51 angiosperm species, all with blooming periods that at least partially overlapped that of *D. californica*, were present at the study sites (Meindl 2009). Within the study populations, common associates included white rushlily (*Hastingsia alba* S. Watson), California bog asphodel (*Narthecium californicum*Baker), Sierra shootingstar (*Dodecatheon jeffreyi* Van Houtte), marsh marigold (*Caltha leptosepala* DC *var. biflora* (DC) G. Lawson), and Bigelow's sneezeweed (*Helenium bigelovii* A. Gray in Torr.).

Flower Visitation and Pollinator Identification

Three observation points were established in each seep in order to monitor pollinator activity. At these points a series of 15-minute surveys were conducted, focusing on 13–17 flowers at one time. Ten surveys (2.5 hours total) were conducted during each day of observation at a field site. Each site was visited three to five times between June 6, 2008 and July 3, 2008 to conduct surveys. Most surveys were made between 10:00 a.m. and 6:00 p.m. In total, 57.5 hr of observations were conducted. Mean flower visitation rates (visits/flower/hour) and the estimated number of visits individual flowers would receive over their lifetimes were calculated for each study site (Meindl 2009). The expected number of visits a flower received over its lifetime was estimated by multiplying the flowering period (in days) by the number of hours in a day pollinators were active (six hr) by the visits/hour calculated for each site. *Darlingtonia californica* pollinators were considered to be active for six hours a day because all visits occurred between 10:30 a.m. and 4:30 p.m. Flower lifespan was determined by monitoring the development of 30 tagged buds at each study site (Meindl 2009).

Following each 15-min census period, five flowers were carefully examined by spreading apart the sepals and petals to check for pollinators already present within the flowers. A total of 1125 flowers were inspected in this way for spiders, spider webs (either inside or outside the flower), fungus gnats, and thrips. Insects were captured by aerial netting or by hand, and identified. For bees collected within the genus *Andrena*, individuals were identified using keys and descriptions from Laberge and Ribble (1975) and compared against previously identified reference specimens in the HSU invertebrate collection. Vouchers of all collected pollinators have been deposited at HSU for future reference.

Pollinator Behavior

To determine if floral visitors carried *D. californica* pollen, each insect collected during surveys (n = 88) was systematically dabbed with a small cube of glycerin jelly containing basic fuchsin stain (Kearns and Inouye 1993). Following pollen removal, the jelly was placed on a microscope slide, melted and covered with a cover slip for analysis. Pollen grains were identified by comparing them to a reference collection prepared from flowers at each site. *Darlingtonia californica* pollen was readily distinguishable from other pollen observed due to its unique morphology, which includes five elongate apertures extending from the grain walls. Pollinators were collected from the flowers of *D. californica*, as well as other coflowering species, to determine which members of the pollinator community carried *D. californica* pollen.

We could not determine the effectiveness of flower visitors directly, but instead recorded how often visitors gathered pollen and contacted stigmas, and how long they spent in flowers. A subset of observed floral visits was filmed with a digital camera. Along with other observed visits, the videos were analyzed to determine if pollinators handled the flowers in a manner that would result in pollination. These observations were also used to indicate whether or not the shape of *D. californica*'s ovary really serves to limit the occurrence of self-pollination.

Pollination Sufficiency and Estimates of Cross-Pollination

Hand-pollinations were performed to estimate pollination sufficiency. At each of the five study sites, 30 flowers were marked as controls and an additional 30 flowers were hand-pollinated. Supplemental pollen was applied twice (separated by one week) to flowers in the hand-pollinated treatment group by rubbing two-three mature anthers directly against stigmatic surfaces, when the appearance of stigmatic exudates indicated receptivity. Pollen used for hand-pollinations was collected from flowers at least five meters away in the same population. Fruit and seed set resulting from unmanipulated control flowers were compared against that of hand-pollinated flowers. If there is no difference in fruit set between these two treatment groups then we can conclude that natural pollination is sufficient, i.e., plants were not pollen-limited.

TABLE 2. FLORAL VISITATION RATES. The mean number of visits a flower was expected to receive per hour and over its lifetime is presented for each study site. Standard error values for mean visits/hour are given in parentheses for each study site.

Site	Visits/hour	Estimated visits/lifetime
CL	0.016 (SE = 0.029)	1.60
SM1	0.041 (SE = 0.042)	2.71
SM2	0.073 (SE = 0.025)	4.84
N17	0.077 (SE = 0.025)	5.08
DF	0.067 (SE = 0.025)	4.42

To gauge relative levels of cross-pollination vs. self-pollination, 30 flowers in each study population were emasculated prior to maturity. Fruits and seeds produced by flowers in the emasculated treatment group were interpreted to be the result of cross-pollination, whereas fruit and seed set by unmanipulated control flowers resulted from both cross-pollination and self-pollination. Thus the contribution of self-pollination to total pollination can be estimated by comparing the fruit and seed set of the emasculated flowers with the fruit and seed set of unmanipulated flowers.

A total of 450 flowers were used for fruit and seed set experiments, with 150 flowers in each of the three treatments: hand-pollinated, emasculated, and unmanipulated. These treatments were spread equally across the five study sites (i.e., 90 flowers at each site in 3 treatment groups of 30). Once fruit maturation began, all treatment flowers were bagged with Reemay® (Fiberweb, TN), a polyester fabric, to ensure seeds were not lost when capsules began to dehisce. Fruit set was determined for each site, as well as the number of seeds produced by each flower that matured a fruit.

Statistical Analyses

A Kruskal-Wallis one-way analysis of variance was used to compare average visitation rates across all sites. A non-parametric test was necessary to analyze visit rate data, as most data points were zeroes and thus the data set could not be adjusted to meet the assumption of normality. Log linear analysis was used to compare the fruit set of the three experimental treatment groups, with treatment, site and the interaction term included in the model. A two-way ANOVA was used to compare seed set across all sites, with treatment and site as the independent variables. Due to a significant interaction term from the two-way ANOVA ($P = 0.041$), separate one-way ANOVAs were run for each site independently using treatment as the independent variable. Post-hoc Tukey-Kramer multiple-comparison tests were used to determine which group means were significantly different from one another. All statistical analyses were performed using NCSS (Hintze 2004).

RESULTS

Flower Visitation and Pollinator Identification

In general, hymenopteran pollinators were abundant at our study sites, represented by eight genera of bees (Meindl 2009). However, *D. californica* received only 38 visits by flying pollinators in 57.5 hr of observations, and nearly all (37) were by a solitary bee, *Andrena nigrihirta*. One visit by a European honeybee (*Apis mellifera*) was also observed. Estimated visit rates varied widely (Table 2), but were not significantly different across the five sites (Kruskal-Wallis $\chi^2 = 5.72$, $P = 0.22$). Based on the average visit rate (pooled data across sites), flowers received 3.9 visits during their entire blooming period. Visits by *A. nigrihirta* were observed throughout the flowering season (6/13/09 through 6/22/09), and multiple visits were observed at each site (Meindl 2009).

Spiders, particularly members of the families Clubionidae, Salticidae, and Theridiidae, were common on flowers at all five study sites, and were active at all hours of the day (Table 3). Whereas a minority of examined flowers contained a spider, the majority showed evidence of spider occupancy (webbing and/or spider present)

TABLE 3. THE PERCENTAGE OF EXAMINED FLOWERS AT EACH STUDY SITE THAT CONTAINED ONE OR MORE OF THE FOLLOWING: THRIPS, SPIDERS, AND SPIDER WEBS (EITHER INSIDE OR OUTSIDE THE FLOWER). A total of 1125 flowers were individually examined (150 at CL, 225 at SM1, 250 at SM2, 250 at N17, and 250 at DF). "Evidence of Spider" column represents the percentage of examined flowers at each site that had a spider and/or webbing present. Only 3/1125 (0.27%) flowers contained one or more fungus gnats.

Site	Web outside flw.	Web inside flw.	Spider present	Evidence of spider	Thrips
CL	38.7	13.3	16.7	48.7	25.3
SM1	48.9	39.6	20	61.8	40.9
SM2	47.2	30.4	24.8	57.2	31.6
N17	69.6	20	34	74	75.6
DF	68.8	30.8	31.2	75.6	58.4
TOTAL	56.2	27.7	26.2	64.8	48.4

(Table 3). Thrips were also present in large numbers at all five sites: nearly half of all examined flowers contained thrips actively foraging for pollen (Table 3). Fungus gnats, while frequently encountered in the seeps, were only observed within *D. californica* flowers three times.

Pollinator Behavior

Individual bees spent up to several minutes within *D. californica* flowers and were found to carry *D. californica* pollen following visits. On average, *A. nigrihirta* foraged on a single *D. californica* flower for approximately two minutes and eight seconds (128 sec ± 12 seconds; n = 14). Eight individuals were collected immediately following visits, and all carried *D. californica* pollen in their scopae. Of these, six carried *D. californica* pollen exclusively while two carried heterospecific pollen as well (Asteraceae). One individual of *A. nigrihirta* was collected in flight (i.e., not on a flower) that carried both *D. californica* and Asteraceae pollen. *Andrena nigrihirta* was the only floral visitor collected that carried the pollen of *D. californica* (Meindl 2009).

Detailed observations of visits by *A. nigrihirta* revealed that the ovary shape of *D. californica* promotes stigma contact by bees both when they enter and exit flowers (Fig. 1). Immediately above the windows (towards the morphological base of the pendant flower), the flower's petals overlap and the underlying petal is appressed to the flared portion of the ovary, which limits the ability of a pollinator the size of *A. nigrihirta* to enter a window and crawl directly up onto the ovary on its way to collect pollen. In between the windows, however, the petals bulge outward (Fig. 2), and it is this space that allows the bee to ascend up to the stamens. This convex portion of each of the five petals is located directly opposite each of the five windows, such that a pollinator enters a window and walks in a straight line across the stigmas and then onto the ovary (directed by the convex portion of the petal). After ascending the ovary, bees were observed to systematically gather pollen before descending down the ovary towards the stigmas. The shape of *D. californica*'s ovary has previously been thought to guide an insect pollinator away from the receptive stigmatic surfaces as it exits the flower, thus preventing self-pollination. However, in exiting the flower, bees were observed to leave in the same fashion as they entered (guided by petal convexities across the stigmas and out one of the windows, thus likely effecting autogamy). This behavioral sequence was exhibited by multiple (n = 27) individuals and was consistent at all sites. These observations, plus evidence that *A. nigrihirta* carried the pollen of *D. californica*, strongly suggest these bees are acting as pollinators.

Pollination Sufficiency and Estimates of Cross-Pollination

Seed production, but not fruit set was pollen-limited. Fruit production by unmanipulated flowers (76%) was not significantly lower than that of hand-pollinated flowers (96%) ($\chi^2 = 3.50$, $P = 0.06$). However, hand-pollinated flowers produced more than twice as many seeds per capsule than unmanipulated flowers at each of the five study sites (Fig. 3).

Self- and cross-pollination both contribute to *D. californica* reproductive success. Emasculated flowers produced fruit and seed at all five sites, indicating that cross-pollination occurred. However, overall fruit set of emasculated flowers (39%) was significantly lower than that of unmanipulated flowers ($\chi^2 = 17.79$, $P < 0.001$), highlighting the importance of autogamous pollen transfer for fruit production. Unmanipulated flowers produced significantly more seeds, on average, than emasculated flowers at SM1 and SM2, but there was no significant difference found between these two treatment groups at the remaining three sites (Fig. 3). Average seed production by unmanipulated flowers was always higher than that of emasculated flowers, regardless of statistical significance, suggesting that cross-pollination cannot account for all of the seeds that were produced. Therefore, fruit and seed production of naturally pollinated flowers were likely the result of both autogamous and xenogamous pollen transfer.

Discussion

Near the summits of Scott Mountain and Mount Eddy in northwestern California, populations of *D. californica* are pollinated by the solitary bee *Andrena nigrihirta*, with additional pollination likely provided by spiders. This conclusion is based on direct observations of floral visits, analysis of bee pollen loads, and the results of our pollination treatments. In particular, even though visit rates were very low, we observed bee visits at all of our sites and estimate that flowers received an average of 4 visits over their extended blooming periods. We could not demonstrate directly that the bees deposited pollen on stigmas, but foragers consistently contacted stigmas when they visited flowers. Moreover, all captured individuals of *A. nigrihirta* carried *D. californica* pollen. Perhaps most importantly, nearly 40% of emasculated flowers produced fruits with seed sets equivalent to controls at three of the five sites. This result indicates substantial cross-pollination, and strongly implicates bees as pollen vectors. However, autogamy must have dominated at our sites because, with one exception (CL), fruit set of unmanipulated flowers was at least twice as high

FIG. 1. Step by step foraging behavior of *A. nigrihirta* on a *D. californica* flower. The bee initially lands on the petals below the windows (a–c) and then enters a window and walks across stigmatic surfaces (d, e). The bee then utilizes the convex portion of one of the five petals to walk onto the ovary and up to collect pollen (f–i). Following pollen collection, the bee uses a petal convexity as before to walk down the ovary, across the stigmas again, then out one of the windows (j–n), before leaving the flower (o, p). The flower is shown in d–m with the front petal removed and half of the two lateral petals removed. Panels f and k show the bee using the convex portion of the petal, which allows the bee to access the stamens.

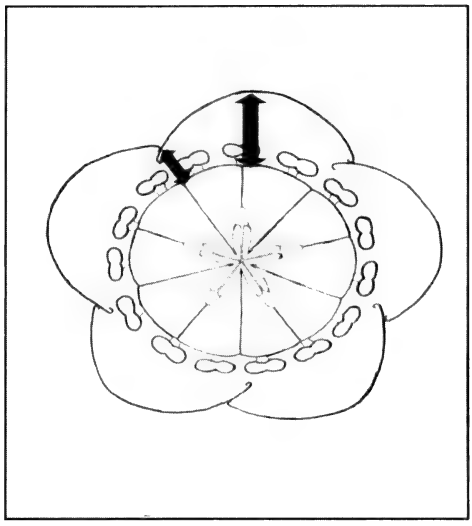

FIG. 2. Interior view of a *D. californica* flower with bottom portion of petals removed. Arrows highlight the distance between the petals and the ovary both immediately above a window (shorter arrow) and in between two adjacent windows (larger arrow). More space is provided for *A. nigrihirta* in between the windows than above them, which encourages the bee to enter a window and then walk across the stigmatic surfaces. The bee then utilizes the convex portion of the petal opposite the window it entered en route to the flower's stamens.

as fruit set of emasculated flowers (Fig. 3). Bees probably accounted for much of this self-pollination because they contacted stigmatic surfaces when they exited flowers after collecting large pollen loads. Spiders, which were abundant on flowers and known to be capable of effecting limited autogamy in *D. californica* (Nyoka 2000), likely further contributed to fruit and seed production via self-pollination. They often constructed webs inside flowers linking anthers and stigmas, and in several instances these webs were completely dusted with pollen. In contrast, spider draglines connecting flowers were very rare at our sites, making it unlikely that spiders contributed significantly to cross-pollination. Pollen-eating thrips were also present in large numbers within flowers, but were rarely seen on stigmas and thus likely played a limited role as pollen vectors. Nyoka and Ferguson (1999) collected fungus gnats carrying *D. californica* pollen in southwestern Oregon, where they may have contributed to seed set. However, although fungus gnats were abundant at our sites, we rarely discovered them inside flowers.

Our findings suggest a resolution of the long-standing mystery surrounding the pollination of *D. californi*ca. Like us, previous workers (Elder 1997; Nyoka 2000) reported high levels of fruit and seed production at their study sites in southwestern Oregon and the northern Sierra Nevada, but rarely or never observed flying pollinators – a discrepancy that led to the provocative hypothesis that omnipresent spiders are the most important pollinators. However, the high levels of pollen limitation observed in this study make it unlikely that spiders are the predominant pollen vectors for *D. californica*, given their abundance on flowers. Although spiders almost certainly contribute to pollen transfer in some degree, we propose instead that *D. californica* is melittophilous, as predicted by Schnell (1976), and specifically that *A. nigrihirta* is responsible for the majority of pollination across its range. Consistent with this view, we now know that *A. nigrihirta* pollinates *D. californica* in northwestern California as well as the northern Sierra Nevada (this study; Rice 2006). The same may be true for populations in southwestern Oregon, where Nyoka (2000) collected a pair of unidentified dark-bodied *Andrena* inside a flower. However, visit rates appear to be very low at all sites, which may partly explain why even observers who spent long periods in populations seldom observed visits. In addition, foragers tend to remain inside flowers for protracted periods (after quickly entering), and usually leave a population after visiting only one or two flowers (G. Meindl, unpublished). The difficulty of detecting these elusive bees is highlighted by the fact that although we spent well over 100 hours at our study sites setting up and monitoring experiments, we observed visits only during our focused census watches (10% of 230 watches). The alternative explanation for the limited number of previously reported visits is that *A. nigrihirta* was either absent or extremely rare at the sites studied by Austin, Elder, Nyoka, and Rice. Although spatial and temporal variation in the local abundance of bee species is well documented (Williams et al. 2001), this explanation begs the question of how to account for the high levels of fruit and seed production documented at these sites. A more parsimonious explanation may be infrequent but effective visits by *A. nigrihirta* coupled with the long period of anthesis of individual *D. californica* flowers. Clearly, additional timed surveys will be needed to document the relative abundance and importance of *A. nigrihirta* as a pollinator across the range of *D. californi*ca.

The relationship between *A. nigrihirta* and *D. californica* appears to be asymmetric, i.e., *D. californica* is specialized on *A. nigrihirta*, but *A. nigrihirta* is a generalist, at least on a broad scale. Across its range, which spans North America and greatly exceeds that of *D. californica*, *A. nigrihirta* is a generalist that has been observed to visit flowers from a diverse array of plants (Laberge and Ribble 1975), including members of Portulacaceae (Motten et al. 1982), Fabaceae (Tepedino et al. 1995), and Ericaceae (Rice 2006),

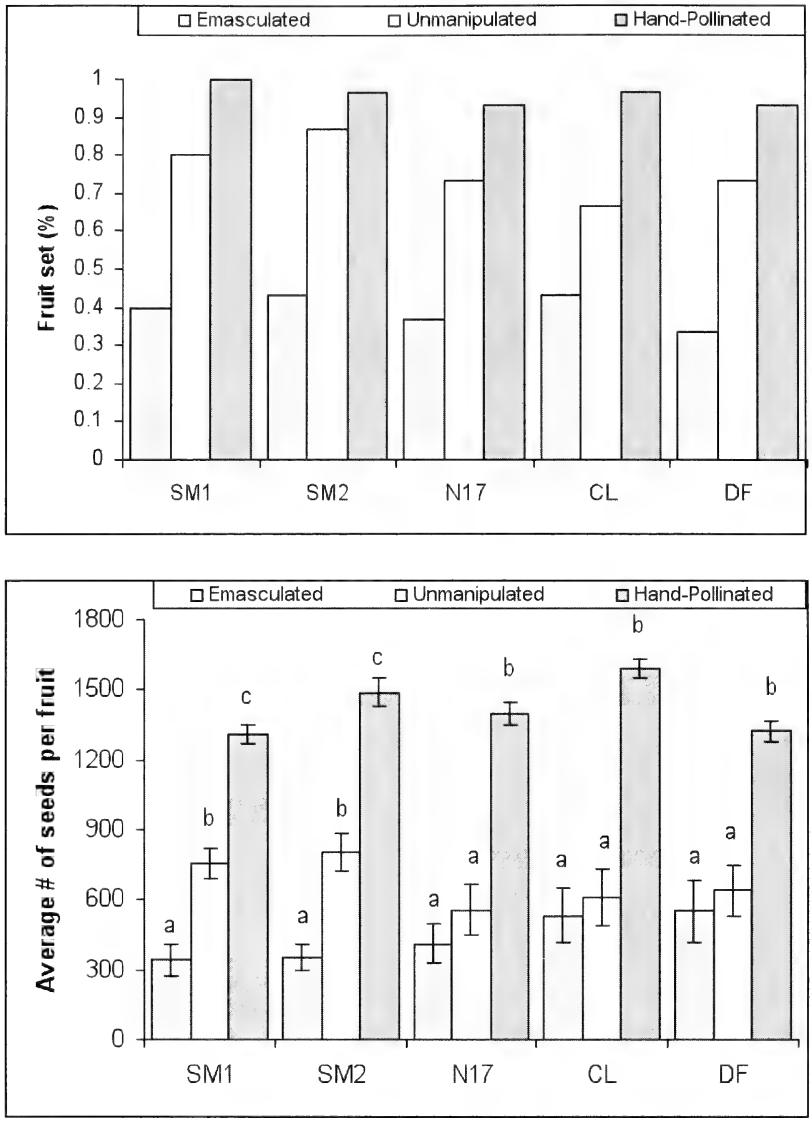

FIG. 3. Fruit and seed production by three treatment groups (emasculated, unmanipulated, and hand-pollinated flowers) at each study site. Top: fruit set (%) of three treatment groups at each field site. Emasculated flowers produced significantly fewer fruits than unmanipulated flowers. No significant difference was found between fruit production of unmanipulated vs. hand-pollinated flowers ($\chi^2 = 3.50$, $P = 0.06$). Bottom: average number of seeds produced per capsule from three treatment groups at each field site. Different letters above bars indicate group means are significantly different (comparisons of group means are only made within sites). Differences in mean seed production between the three treatment groups were evident at all 5 sites (SM1: $F_{2,63} = 65.35$, $P < 0.001$; SM2: $F_{2,65} = 53.76$, $P < 0.001$; CL: $F_{2,59} = 52.49$, $P < 0.001$; N17: $F_{2,58} = 46.31$, $P < 0.001$; DF: $F_{2,57} = 24.19$, $P < 0.001$).

along with *D. californi*ca. Three individuals of *A. nigrihirta* were collected during this study that carried both *D. californica* and Asteraceae pollen, indicating that *A. nigrihirta* is utilizing floral resources from multiple species of flowering plants. While asymmetrical species interactions are known to be common in ecological networks (Vazquez et al. 2007), it is unclear why *D. californica* relies so heavily on *A. nigrihirta* for pollination, considering the abundance of other bee species at our study sites.

Despite visits by *A. nigrihirta* being rare, the morphometric fit between bee and flower appears strong. While bumblebees were among the most abundant pollinators active at our study sites, their large body size prevented them from utilizing *D. californica* as a floral resource (G. Meindl, unpublished). Likewise, honeybees also have difficulty entering and handling the flowers (Rice 2006). *Andrena nigrihirta* was able to enter the small windows of *D. californica* flowers quickly and efficiently, and proved to be of an

ideal size to contact stigmas, climb onto the ovary beneath the petal convexities and gather pollen from the flower's anthers. Paradoxically, several other bee species collected at our field sites were of similar size to *A. nigrihirta* (e.g., other *Andrena* spp., *Osmia* spp., and *Lasioglossum* spp.; G. Meindl, unpublished), yet only *A. nigrihirta* was observed to forage on *D. californica* flowers. Further studies are needed to characterize the relationship between *D. californica* and its bee pollinators, and to determine why visits are made predominantly by *A. nigrihirta* and not by other similarly sized bee species. However, the preference shown to the flowers of *Darlingtonia* at our field sites in northern California, along with the morphological match between bee and flower, suggest that *A. nigrihirta* and *D. californica* have an established relationship. The detailed accounts of floral visitation in this study, combined with the results of pollination treatments, provide sound evidence that *D. californica* produces melittophilous flowers that are effectively, though rarely, pollinated by the solitary bee *A. nigrihirta*.

There are several interesting ecological questions that have yet to be considered regarding *D. californica* pollination. For instance, why are visits by bees so infrequent? How do spiders occupying *D. californica* flowers interact with bees? Does the presence of spiders within flowers deter visitation by bees, or do bees frequently fall victim to lurking spiders, and what bearing does this have on *D. californica* reproductive success? Over the course of floral observations conducted in this study, *A. nigrihirta* was seen "buzzing" flowers, i.e. approaching flowers but not entering them, more frequently than entering flowers (37 flowers visited, 50 flowers buzzed). While this "buzzing" behavior could be males searching flowers for females, other explanations are also possible. For example, this behavior could be the result of floral marking by bees, which may be done to alert future visitors of resource availability (Schmitt and Bertsch 1990; Goulson et al. 2001), or may also be the result of altered foraging behavior caused by the presence of flower-occupying spiders (Bruce et al. 2005; Goncalves-Souza 2008). It is also unclear how floral form influences pollination by bees vs. spiders, i.e., do the same floral traits that promote pollen deposition on stigmatic surfaces by bees (shape of ovary, etc.) also promote pollen deposition by spiders, or should we expect divergence of floral morphology in *D. californica* populations that occur in areas where *A. nigrihirta* is absent over time? As we seek to explain the adaptive significance of *D. californica*'s floral traits, we need to understand, in greater detail, the effects of these multi-species interactions on trait selection.

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A MORPHOMETRIC ANALYSIS OF VARIATION BETWEEN *ELYMUS ALASKANUS* AND *ELYMUS VIOLACEUS* (POACEAE): IMPLICATIONS FOR RECOGNITION OF TAXA

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ABSTRACT

The aim of this study was to clarify the relationships between *Elymus alaskanus* and *E. violaceus* in northwest North America. We performed a morphological and biogeographic analyses of ca. 300 widely distributed herbarium specimens. Following a univariate analysis of morphological characters used in contemporary treatments, we found no clear character, or combination of characters, that differentiates unambiguously among the taxa at the specific level. However, glume and lemma trichome length reliably separated *E. alaskanus* subsp. *hyperarcticus* from other taxa. Specimens could not be differentiated at the specific level by habitat preferences or geographic distribution as described in the most current treatments. Further, principal components analysis and cluster analysis were unable to reliably segregate specimens into groups. Discriminant analysis reliably grouped *E. violaceus* and *E. alaskanus* subsp. *hyperarcticus*, but not *E. alaskanus* specimens. In the development of a relevant treatment for *E. alaskanus* and *E. violaceus*, we recommend that (i) *E. violaceus* be treated as a subspecies of *E. alaskanus* and called *E. alaskanus* subsp. *latiglumis*, and (ii) *E. alaskanus* subsp. *alaskanus* and *E. alaskanus* subsp. *hyperarcticus* continue to be recognized at the subspecific level.

Key Words: British Columbia, *Elymus alaskanus*, *Elymus violaceus*, taxonomy, *Triticeae*.

Delineation of taxa within grass tribe *Triticeae* (Poaceae) has been complicated and controversial (Dewey 1983a; Barkworth 1992; Zhang et al. 2000; Barkworth et al. 2007), with disagreement over taxonomic treatments at the generic and specific level (Hitchcock 1951; Tzvelev 1976; Löve 1980a, b; Melderis 1980; Dewey 1983b, 1984; Barkworth 1992; Stewart and Barkworth 2001; Barkworth et al. 2007). The development of a stable nomenclature for the tribe has been inhibited by the morphological complexity of the group and lack of widely accepted criteria for the most appropriate taxonomic treatment (Barkworth 1992).

Elymus L., within the *Triticeae*, has the most species and widest distribution as interpreted by Dewey (1984), Löve (1984) and Barkworth et al. (2007). It occurs worldwide in non-tropical regions and includes approximately 150 north-temperate perennial species (Dewey 1984; Zhang et al. 2000; Sun et al. 2006b; Barkworth et al. 2007). In the northwest North American province of British Columbia, Canada, there are twelve recognized species, of which *Elymus alaskanus* (Scribn. & Merr.) Á. Löve and *E. violaceus* (Hornem.) J. Feilberg are poorly resolved. *Elymus* species inhabit diverse ecological niches, including forests and forest edges, mountain slopes and valleys, semi-deserts and grasslands (Sun et al. 2006b). *Elymus* morphology varies widely within and among species because of introgression, the ability of species to form intra- and interspecific fertile hybrids and the polyploid origin of the genus (Sun and Li 2005; Barkworth et al. 2007). Additionally, morphological variability among species is partially under environmental control (Sun and Li 2005; Sun et al. 2006a; Barkworth et al. 2007). The high levels of variability observed in morphological traits are consistent with the genetic variability observed in molecular studies (Díaz et al. 1999; Zhang et al. 2000, 2002; Sun and Salomon 2003).

Alaskan wheatgrass, *Elymus alaskanus* and Arctic wheatgrass, *Elymus violaceus* are perennial, allotetraploid species (**StStHH**, $2n = 4x = 28$) that illustrate the taxonomic difficulty of *Elymus* (Zhang et al. 2000; Sun and Salomon 2003; Barkworth et al. 2007). Previously, this species complex has been placed in several different taxa (cf. Hitchcock 1951; Welsh 1974; Löve 1984; Baum et al. 1991; Cody 1996; Barkworth et al. 2007) (Table 1). Morphological similarity between *Elymus alaskanus* and *Elymus violaceus* has lead to contradictory taxonomic conclusions, and taxonomists are not in agreement on whether or not the two are separate species (Zhang et al.

2000; Stewart and Barkworth 2001; Sun et al. 2006a; Barkworth et al. 2007). The issue of distinguishing the two taxa morphologically is illustrated in the two comprehensive treatments covering British Columbia: *The Flora of North America* (FNA) *Volume 24* (Barkworth et al. 2007) and *The Illustrated Flora of British Columbia Volume 7* (Stewart and Barkworth 2001). Stewart and Barkworth (2001), recognize only one member at the specific level, *E. alaskanus* (Scribn. & Merr.) Á. Löve subsp. *latiglumis* (Scribn. & J.G. Sm.) Á. Löve (=*E. violaceus*), whereas Barkworth et al. (2007), recognize two species, *Elymus alaskanus* and *Elymus violaceus*. The treatment in the FNA (Barkworth et al. 2007), in accordance with Hultén (1968), asserts that *E. alaskanus* is differentiated from *E. violaceus* in having relatively shorter glumes than *E. violaceus* (Barkworth et al. 2007). Those of *E. alaskanus* are said to be ⅓ to ⅔ as long as the adjacent lemmas, and those of *E. violaceus* ¾ to equal to the lemma length (Barkworth et al. 2007). Following Löve (1984) and Cody (1996), Barkworth et al. (2007) further divide *E. alaskanus* into subspecies, naming plants with relatively glabrous glumes and lemmas as *E. alaskanus* subsp. *alaskanus*, and those with glumes and lemmas covered densely by trichomes as *E. alaskanus* subsp. *hyperarcticus* (Polunin) Á. Löve & D. Löve. Both taxa are mostly arctic or alpine (sometimes subalpine) species with a northern circumpolar distribution. However, the more restricted range of *E. alaskanus* is thought to distinguish it from *E. violaceus* (Barkworth et al. 2007). *Elymus alaskanus* grows across the high arctic of North America to eastern Russia, through Siberia, Alaska, northern USA and Greenland (Zhang et al. 2000; Sun and Salomon 2003), but according to the FNA distribution maps is almost absent from British Columbia (Barkworth et al. 2007: 326). The distribution of *E. violaceus* extends from Alaska across arctic Canada to Greenland and south in the Rocky Mountains to southern New Mexico (Barkworth et al. 2007). In western North America *E. alaskanus* is often associated with valleys and flat sites in low-competition habitats such as limestone outcrops, scree, moraines and dry meadows (Zhang et al. 2000; Barkworth et al. 2007), whereas *E. violaceus* favours calcareous or dolomitic rock in arctic, subalpine and alpine habitats. In general, *E. alaskanus* is thought to be found at lower elevations than *E. violaceus* (Barkworth et al. 2007).

The aim of this study is to clarify the relationships between *E. alaskanus* and *E. violaceus* by performing morphological and biogeographic analyses of herbarium specimens collected from a broad geographic range in northwest North America, and to answer two questions. 1) Can *E. alaskanus* and *E. violaceus* be regarded as separate species in British Columbia and adjacent regions? And if so, 2) what morphological, geographical and habitat characters can be used to discriminate between the species? Our overall objective is to contribute to the development of a single taxonomic treatment for *E. alaskanus* and *E. violaceus* in northwest North America and advance our understanding of these taxa over their broader ranges. Increased knowledge of the relationship among entities will be especially useful in British Columbia because of the widespread geographic overlap of the two species and current disagreement over their treatment within the province (e.g., Stewart and Barkworth 2001; Barkworth et al. 2007).

Methods

Nomenclatural Considerations

Two sets of infraspecific taxa can be considered in Table 1, those in the "*boreale/alaskanus*" complex and those in the "*latiglumis/violaceus/hyperarcticus*" complex. When considering the infraspecific taxa from the boreale/alaskanus column (Table 1), we regard *E. alaskanus* and *E. alaskanus* subsp. *borealis* (Turcz.) Á. Löve & D. Löve as constituting the same taxon because in general taxonomists agree that differences between the potential subspecies do not warrant recognition (Stewart and Barkworth 2001; Barkworth et al. 2007). Hultén (1968) and Welsh (1974) recognized three subspecies within *Agropyron boreale* Drobow, as did Löve (1984) and Cody (1996), but they placed the subspecies in *Elymus*. Taxonomists placing the members of this nomenclatural set in *Elymus* had to change the specific epithet used from "*boreale*" to "*alaskanus*" in order to conform with the rules of the International Code of Botanical Nomenclature (McNeill et al. 2006). We followed Barkworth et al. (2007) who differed from pre-existing treatments in combining these two infraspecific taxa into a single taxon, which, according to the rules of priority, were called *Elymus alaskanus* subsp. *alaskanus*. The fundamental question concerning the treatment of "*latiglumis*" and "*violaceus*" concerns the appropriate names to be applied. Scribner and Smith (1897) originally named these plants *Agropyron violaceum* (Hornem.) Lange var. *latiglume* Scribn. & J. G. Sm. Their description provided a brief description of the new variety, but did not state how the entity differed from var. *violaceum*. Generally, taxonomists agree that "*latiglumis*" and "*violaceus*" refer to the same taxon (Stewart and Barkworth 2001; Soreng et al. 2003; Barkworth et al. 2007), with the exception of Löve (1984) who applied separate names, but this compendium of taxonomic groups within the *Triticeae* was based on

TABLE 1. HISTORICAL NOMENCLATURE OF THE *ELYMUS ALASKANUS* AND *E. VIOLACEUS* COMPLEXES IN NORTH AMERICA (MODIFIED FROM BARKWORTH 1997).

Reference	Entity				
	"boreale"	*"alaskanus"*	*"hyperarcticus"*	*"latiglumis"*	*"violaceus"*
Turczaninow (1856)	*Triticum boreale*	—	—	—	—
Scribner and Smith (1897)	—	—	—	*Agropyron violaceum* var. *latiglume*	—
Scribner (1900)	*Elymus borealis*	—	—	—	—
Rydberg (1909)	—	—	—	*Agropyron latiglume*	—
Scribner and Merrill (1910)	—	*Agropyron alaskanum*	—	—	—
Drobow (1916)	*Agropyron boreale*	—	—	—	—
Nevski (1934)	*Roegneria borealis*	—	—	—	—
Polunin (1940)	—	—	*Agropyron violaceum* var. *hyperarcticum*	—	—
Hitchcock (1951)	—	—	—	*Agropyron latiglume*	—
Beetle (1952)	—	—	—	*Roegneria latiglumis*	—
Löve & Löve (1956)	—	—	*Roegneria borealis* subsp. *hyperarctica*	—	—
Polunin (1959)	*Agropyron boreale*	—	—	—	*Agropyron violaceum*
Hultén (1968)	*Agropyron boreale* subsp. *boreale*	*Agropyron boreale* subsp. *alaskanum*	*Agropyron boreale* subsp. *hyperarcticum*	—	*Agropyron violaceum* subsp. *violaceum*
Hitchcock (1969)	—	—	—	*Agropyron caninum* subsp. *majus* var. *latiglume*.	—
Hitchcock and Cronquist (1973)	—	—	—	*Agropyron caninum* subsp. *majus* var. *latiglume*.	—
Welsh (1974)	*Agropyron boreale* var. *boreale*	*Agropyron boreale* var. *alaskanum*	*Agropyron boreale* var. *hyperarcticum*	*Agropyron caninum* var. *latiglume*	—
Löve & Löve (1976)	*Elymus alaskanus* subsp. *borealis*	—	—	—	—
Tzvelev (1976)	—	—	*Elymus sajanensis* subsp. *hyperarcticus*	—	—
Scoggan (1978)	—	—	—	*Agropyron trachycaulum* var. *latiglume*	—
Porsild and Cody (1980)	—	—	*Agropyron violaceum* var. *hyperarcticum*	—	*Agropyron violaceum* subsp. *violaceum*
Dore and McNeill (1980)	—	—	—	—	*Agropyron violaceum*
Moss (1983)	—	—	—	—	*Agropyron violaceum*
Löve (1984)	*Elymus alaskanus* subsp. *borealis*	*Elymus alaskanus* subsp. *alaskanus*	*Elymus alaskanus* subsp. *hyperarcticus*	*Elymus alaskanus* subsp. *latiglumis*	*Elymus trachycaulus* subsp. *violaceus*
Baum et al. (1991)	*Roegneria borealis*		*Roegneria borealis* or *R. borealis* var. *hyperarctica*	*Roegneria latiglumis*	—
Cody (1996)	*Elymus alaskanus* subsp. *borealis*	*Elymus alaskanus* subsp. *alaskanus*	*Elymus alaskanus* subsp. *hyperarcticus*	—	*Elymus trachycaulus* subsp. *violaceus*

TABLE 1. Continued.

Reference	Entity				
	"boreale"	*"alaskanus"*	*"hyperarcticus"*	*"latiglumis"*	*"violaceus"*
Stewart and Barkworth (2001)	—	—	—	*Elymus alaskanus* subsp. *latiglumis*	—
Barkworth et al. (2007)	—	*Elymus alaskanus* subsp. *alaskanus*	*Elymus alaskanus* subsp. *hyperarcticus*	—	*Elymus violaceus*
Harrison and Hebda (this study)	—	*Elymus alaskanus* subsp. *alaskanus*	*Elymus alaskanus* subsp. *hyperarcticus*	*Elymus alaskanus* subsp. *latiglumis*	—

names, not the plants themselves. The name *Agropyron violcaeum* var. *latiglume*, as it appears on the holotype for this entity, was called *Elymus violaceus* by Barkworth et al. (2007) in the Flora of North America not to reflect a new entity but to include *E. alaskanus* subsp. *latiglumis* [=*Agropyron latiglume* Rydb.]. Here we regard *E. violaceus* and *E. alaskanus* subsp. *latiglumis* as synonyms following the work of contemporary taxonomists (Stewart and Barkworth et al. 2001; Soreng et al. 2003; Barkworth et al. 2007).

Sampling and Measurements

Herbarium specimens from the Royal BC Museum (V), the University of British Columbia (UBC), the Canadian Museum of Nature (CAN) and the United States National Herbarium (US) were used as the basis for this study (Appendix 1). All specimens included in the analysis evidently belonged in the taxa of interest, thus none were disqualified. Potential hybrid specimens (i.e., intermediate morphologies) were not excluded from the analysis because doing so could potentially create artificial groupings. Specimens retaining historical nomenclature had current names applied to them following the Flora of North America (FNA) (Barkworth et al. 2007) and were divided into three categories (1) *E. alaskanus sensu stricto* (includes specimens named *E. alaskanus* and *E. alaskanus* subsp. *alaskanus*) (2) *E. alaskanus* subsp. *hyperarcticus* and (3) *E. violaceus*. A preliminary analysis of specimens revealed that identifiers correctly applied the name *E. a.* subsp. *hyperarcticus* to specimens with hairier glumes and lemmas as described in the FNA (Barkworth et al. 2007). Hence, we are confident that our analysis of the broader taxonomic group *E. alaskanus* did not include specimens of *E. a.* subsp. *hyperarcticus*. From herein we will refer to specimens of *E. alaskanus* and *E. alaskanus* subsp. *alaskanus* collectively as *E. alaskanus sensu stricto* (*s.s.*) and specimens including all three taxa as *E. alaskanus sensu lato* (*s.l.*). In total, 109 *E. alaskanus s.s.*, 18 *E. alaskanus* subsp. *hyperarcticus* and 169 *E. violaceus* specimens were included in the analysis. Plants originated from the northwest continental United States, Alaska and Canada (Table 2). Type specimens from CAN and US were examined separately and included (1) *Agropyron alaskanum* Scribn. and Merr. (Contrib. U.S. Natl. Herb. 13: 85. 1910. Type: United States: Alaska. Circle City. 18 Aug. 1899. *W.H. Osgood s.n.* [holotype: US]); (2) *Agropyron violaceum* var. *latiglume* Scribn. and J.G. Sm. (U. S. Dept. Agric. Div. Agrost. Bull. 4: 30. 1897. Type: United States: Montana. Gallatin Co., Lone Mountain, *Tweedy 1011* [holotype: US]); (3) *Agropyron violaceum* var. *hyperarcticum* Polunin (Bull. Natl. Mus. Canada 92 (Biol. Ser.

TABLE 2. GEOGRAPHIC ORIGIN AND NUMBER OF *ELYMUS ALASKANUS SENSU STRICTO* (N = 110), *E. ALASKANUS* SUBSP. *HYPERARCTICUS* (N = 18) AND *E. VIOLACEUS* (N = 169) SPECIMENS EXAMINED FOR MORPHOLOGICAL ANALYSIS IN THIS STUDY. AK = Alaska, AB = Alberta, BC = British Columbia, MT = Montana, NU = Nunavut, NWT = Northwest Territories, ON = Ontario, QC = Quebec, UT = Utah, WA = Washington, YT = Yukon Territory.

	AK	AB	BC	MT	NU	NWT	ON	QC	UT	WA	YT
E. alaskanus	9	2	38	—	—	37	—	1	—	—	23
E. a. subsp. hyperarcticus	7	—	—		1	4	—	—	—	—	6
E. violaceus	3	4	134	1	—	8	1	—	1	2	15

24): 95. 1940. Type: Canada: Nunavut, Baffin Is., Arctic Bay, 9 Sept. 1936. *N. Polunin 2531* [isotype: CAN]).

We used 22 morphological characters for analyses (Table 3). All measurements of glume and lemma characteristics were made under 10× magnification to the nearest 0.1 mm using an ocular micrometer. Blade length and width, spikelet, culm, and inflorescence length were measured with a line ruler to the nearest 1mm. Spikelets were selected from the middle of the inflorescence and the glume and lemma were chosen from the same spikelet. All lemmas, regardless of their stage of development, were counted. Ratios between lower glume and spikelet length, the lower glume and lemma length, and between glume margin width at widest point to total glume length were calculated. Measurements of both glumes and lemmas did not include the awns which were considered separately.

Habitat, elevation and geographical information were recorded from herbarium sheets. All specimens from Alaska, Alberta, British Columbia, Northwest Territories, Nunavut and Yukon with sufficient geographic information on herbarium labels were mapped using ArcView 9.3 (2008).

Morphological Analysis

Univariate analysis. We used univariate analyses to examine the effectiveness of using glume to lemma ratio as the key diagnostic character separating *E. alaskanus s.l.* and *E. violaceus* (as currently done in the Flora of North America volume 24, Barkworth et al. 2007). We also considered the effectiveness of using lemma and glume trichome length to identify *E. alaskanus* subsp. *hyperarcticus*. Data did not meet assumptions for normality (Shapiro-Wilk test statistic) and homogeneity of variance (plot of residuals versus fits), thus a Kruskall-Wallis test of the equality of medians was performed as a nonparametric alternative to analysis of variance (ANOVA). Boxplots were used for visual comparison of these traits. Additionally, we took as a subset of specimens, those identified by M. Barkworth (Intermountain Herbarium, Utah State University), to analyze differences in glume to lemma ratio among taxa while reducing the variation in the interpretation of the diagnostic criteria. This subset of data met assumptions of normality and equal variance; thus ANOVA was performed and boxplots were created to investigate differences among groups. All univariate analyses were computed with Minitab (2007). Null hypotheses were rejected at $P < 0.05$. Lower glume to lower lemma measurements and ratios of type specimens from CAN and US were examined separately.

Multivariate analysis. Multivariate analyses tests included principal components analysis, discriminant analysis and cluster analysis. Correlation matrices were constructed to investigate linear relationships between morphological variables using Pearson's product moment correlation. Lower glume length, lower lemma length and spikelet length were excluded from multivariate analyses because they were components of computed ratios and elevation was excluded because a preliminary analysis indicated it varied with latitude. Because tests require that all observations are present for all cases, we excluded anther length which had a high proportion of missing values. In total, 286 specimens were used. Morphological characters included in these analyses are reported in Table 3.

We used principal components analysis (PCA) to identify morphological characters that contributed most to the variation among specimens and to characterize the pattern of trait relationships between *E. alaskanus s.s., E. alaskanus* subsp. *hyperarcticus* and *E. violaceus*. Eighteen variables were included in the anlaysis. PCA was performed using a correlation matrix and six principal components were computed. Factor scores were used in subsequent ANOVAs to test the significance of factors among the taxa.

To assess how well trait measures could be used to correctly classify plants into taxonomic groups, we used discriminant analysis. For this analysis a quadratic discriminant function with fits was applied. To determine if our observations could be segregated into groups that were not defined in advance we used cluster analysis. A dendrogram was produced using single linkage and Euclidean distance, with variables standard-

TABLE 3. CHARACTERS MEASURED OR RECORDED FOR ANALYSIS. *Characters used in Principal Components Analysis (PCA), discriminant analysis and cluster analysis. †Margin to glume length ratio excluded from discriminant analysis because it was highly correlated with other predictors in *E. alaskanus* subsp. *hyperarcticus*.

Character	Description
Culm	
Culm length*	Length (cm) from below the inflorescence to culm base
Blade	
Blade length*	Length (cm) of longest blade
Blade width*	Width (cm) of widest point of longest blade
Inflorescence	
Inflorescence length*	Length (cm) of longest inflorescence; without awns
Inflorescence width*	Width (mm) of widest point of longest inflorescence
Spikelet	
Spikelet length	Length (mm); awnless; spikelet from mid-inflorescence
Spikelet width*	Width (mm) at widest point; spikelet from mid-inflorescence
Glume	
Lower glume length	Length (mm) of lower glume; awnless
Lower glume width *	Width (mm) at widest point of lower glume
Glume margin width *	Width (mm) of glume margin
Glume trichome length*	Length (mm) of glume trichomes
Glume veins*	Number of glume veins
Glume awn length*	Length (mm) of glume awn
Lemma	
Lower lemma length	Length (mm) of lower lemma ; awnless
Lower lemma width*	Width (mm) of lower lemma at widest point
Lemma awn length*	Length (mm) of awn length of lower lemma
Lemma trichome length*	Length (mm) of lemma trichomes
Anther length	Length (mm) of anthers
Floret number*	Total number of florets within spikelet; all stages of development
Ratios	
Margin/glume length*†	Width of glume margin at widest point to total glume length
Glume/spikelet*	Lower glume length to spikelet length
Glume/lemma*	Lower glume length to lower lemma length
Other	
Habitat	From herbarium sheet
Location	From herbarium sheet
Elevation	From herbarium sheet

ized. All multivariate analyses were computed with Minitab (2007).

Biogeographic analysis. To determine if differences in elevation exist among *E. alaskanus s.s.*, *E. alaskanus* subsp. *hyperarcticus* and *E. violaceus*, specimens were placed in latitude categories: (1) all latitudes (2) ≥60°N (true arctic) (3) 55°–60°N (transition-boreal) (4) <55°N (southern alpine). Data in the first three groups did not meet assumptions of normality or homogeneity of variance, thus a Kruskall-Wallis test was performed to test for differences in elevation among taxa. Data in group 4 met parametric assumptions and ANOVA was performed. For the habitat analysis, all specimens with adequate information on herbarium labels (Appendix 1) were classified into two categories (1) rocky habitats or (2) valleys/flat areas and a chi-square test was performed to look at associations between habitat type and taxa.

RESULTS

Morphological Analysis

Univariate analysis. All morphological characters generally had overlapping ranges (Table 4). Taxa differed in glume to lemma ratio (Kruskall-Wallis, df = 2, $P < 0.001$ adjusted for ties; Fig. 1). A subset of specimens, those identified by Barkworth, also differed in glume to lemma ratio among taxa (ANOVA, $F_{(2,113)} = 43.15$, $P < 0.001$; $R^2 = 0.423$; Fig. 2). Following ANOVA, pairwise comparisons among taxa (Tukey 95% simultaneous confidence intervals) showed no significant differences between *E. alaskanus s.s.* and *E. alaskanus* subsp. *hyperarcticus*, but did find that *E. alaskanus* subsp. *hyperarcticus* is significantly different from *E. violaceus*, and *E. alaskanus s.s.* is different from *E. violaceus*. Highly significant differences among taxa were detected for both lemma trichome length (Krus-

TABLE 4. MEAN, STANDARD DEVIATION AND RANGE (IN PARENTHESIS) FOR 22 TAXONOMIC TRAITS OF *ELYMUS ALASKANUS SENSU STRICTO, E. ALASKANUS* SUBSP. *HYPERARCTICUS* AND *E. VIOLACEUS*.

Variable	*E. alaskanus*	*E. a. subsp. hyperarcticus*	*E. violaceus*
Culm length (cm)	33.1 ± 1.3 (10.0–69.0)	24.26 ± 1.98 (12.0–45.1)	31.20 ± 1.17 (10.0–77.5)
Blade width (cm)	0.3 ± 0.02 (0.1–0.9)	0.3 ± 0.03 (0.1–0.5)	0.3 ± 0.01 (0.1–1.9)
Blade length (cm)	8.8 ± 0.4 (2.1–20.0)	7.4 ± 0.6 (4.2–14.0)	7.8 ± 0.4 (1.9–41.0)
Inflorescence length (cm)	7.5 ± 0.2 (3.2–15.0)	6.4 ± 0.5 (3.5–10.0)	6.9 ± 0.2 (3.1–15.0)
Inflorescence width (cm)	0.5 ± 0.02 (0.3–2.0)	0.6 ± 0.4 (0.4–1.1)	0.6 ± 0.01 (0.3–1.8)
Spikelet length (mm)	12.6 ± 0.2 (7.7–20.7)	11.4 ± 0.4 (8.8–14)	12.3 ± 0.1 (7.8–20.7)
Spikelet width (mm)	2.1 ± 0.05 (0.4–3.9)	2.2 ± 0.09 (1.8–3.1)	2.2 ± 0.03 (1.1–3.5)
Lower glume length (mm)	6.9 ± 0.2 (2.4–13.5)	5.9 ± 0.2 (5.0–7.4)	8.2 ± 0.1 (4.9–13.0)
Lower glume width (mm)	1.5 ± 0.03 (0.6–2.8)	1.4 ± 0.08 (0.7–1.9)	1.7 ± 0.02 (1.0–2.5)
Lower glume awn length (mm)	0.8 ± 0.08 (0.0–6.5)	0.7 ± 1.3 (0.2–2.6)	0.8 ± 0.04 (0.0–4.0)
Number of glume veins	2–5	2–3	2–5
Width of lower glume margin at widest point (mm)	0.4 ± 0.1 (0.0–0.8)	0.4 ± 0.02 (0.2–0.6)	0.5 ± 0.01 (0.1–1.0)
Lower lemma length (mm)	9.1 ± 0.1 (5.8–14.0)	8.7 ± 0.3 (6.9–12.0)	8.8 ± 0.08 (6.4–12.0)
Lower lemma width (mm)	1.7 ± 0.03 (0.6–2.5)	1.8 ± 0.06 (1.2–2.2)	1.7 ± 0.02 (1.0–2.5)
Lower lemma awn length (mm)	2.1 ± 0.2 (0.0–7.5)	3.3 ± 0.4 (1.0–6.2)	1.0 ± 0.08 (0.0–9.9)
Number of florets	2–6	1–4	1–6
Lower glume trichome length (mm)	0.04 ± 0.008 (0.0–0.3)	0.2 ± 0.3 (0.0–0.6)	0.007 ± 0.002 (0.0–0.3)
Lower lemma trichome length (mm)	0.2 ± 0.01 (0.0–0.6)	0.4 ± 0.02 (0.2–0.6)	0.2 ± 0.01 (0.0–1.0)
Anther length (mm)	1.2 ± 0.02 (0.7–2.1)	1.2 ± 0.05 (1.0–1.7)	1.1 ± 0.02 (0.5–1.8)
Lower glume length/ spikelet length	0.6 ± 0.01 (0.2–0.9)	0.5 ± 0.02 (0.4–0.7)	0.7 ± 0.008 (0.3–1.03)
Lower glume length/lower lemma length	0.8 ± 0.01 (0.4–1.2)	0.7 ± 0.02 (0.5–0.8)	0.9 ± 0.009 (0.7–1.5)
Glume margin width at widest point/ lower glume length	0.7 ± 0.1 (0.4–1.4)	0.7 ± 0.04 (0.3–1.0)	0.7 ± 0.01 (0.28–1.0)

kall-Wallis, df = 2, $P < 0.001$ adjusted for ties; Fig. 3) and glume trichome length (Kruskall-Wallis, df = 2, $P < 0.001$ adjusted for ties; Fig. 4). Type specimen measurements indicate that *Elymus violaceus* (=*Agropyron violaceum* var. *latiglume*) had a glume to lemma ratio of 0.91, *Elymus alaskanus* subsp. *alaskanus* (=*Agropyron alaskanum*) had a ratio of 0.59, and *Elymus alaskanus* subsp. *hyperarcticus* (=*Agropyron violaceum* var. *hyperarcticum*) a ratio of 0.76.

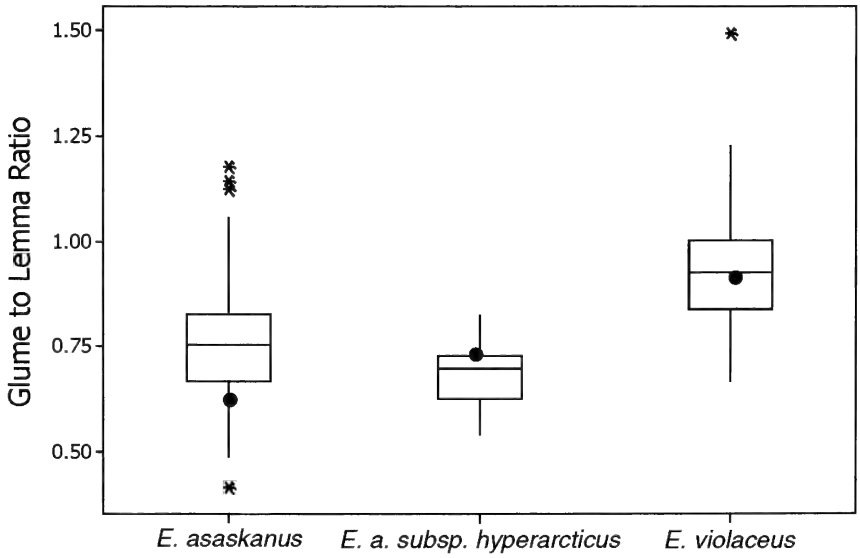


FIG. 1. Glume to lemma ratio for *Elymus alaskanus sensu stricto* (n = 110), *E. alaskanus* subsp. *hyperarcticus* (n = 18) and *E. violaceus* (n = 169). Glume to lemma ratio for type specimens *Elymus violaceus* (=*Agropyron violaceum* var. *latiglume*), *Elymus alaskanus* subsp. *alaskanus* (=*Agropyron alaskanum*), and *Elymus alaskanus* subsp. *hyperarcticus* (=*Agropyron violaceum* var. *hyperarcticum*) indicated by ● symbol.

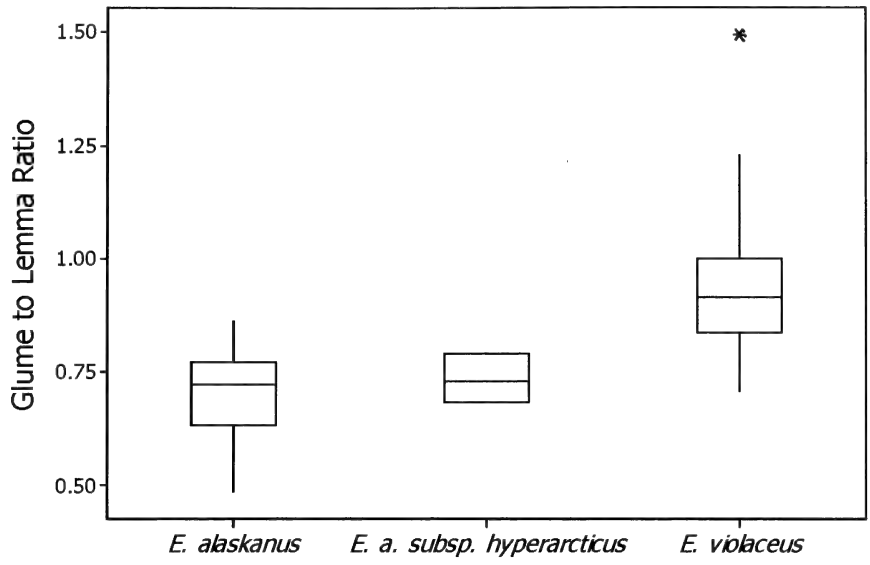


FIG. 2. Glume to lemma ratio for specimens of *Elymus alaskanus sensu stricto* (n = 32), *E. alaskanus* subsp. *hyperarcticus* (n = 3) and *E. violaceus* (n = 81) identified by Barkworth.

Multivariate analysis. Correlations among morphological characters used in the multivariate analysis ranged from 0.021 to 0.8, thus none were excluded from the analysis. Five principle components (PC) had eigenvalues >1 and the first three components accounted for 47% of the variation in the data set (Table 5; Fig. 5). The first principle component (PC1) accounted for 20% of the total variance, with the lower glume width and glume length to lemma length ratio and lower lemma width having the highest coefficients, and all loading positively on PC1. In contrast, blade length, lemma awn length and culm length loaded negatively on PC1. PC2 accounted for 15.7% of the total variance and reflected increased inflorescence length, blade length and culm length, but decreased trichome lengths of both glumes and lemmas. Spikelet width, lower lemma width and glume trichome length loaded negatively on PC3 and glume to spikelet length ratio and glume trichome length loading positively.

An ANOVA using PC1 scores confirmed differences among taxa (ANOVA, $F_{(2,283)} = 28.65$, $P < 0.001$; $R^2 = 0.168$), with *E. violaceus* having significantly larger PC1 scores than either

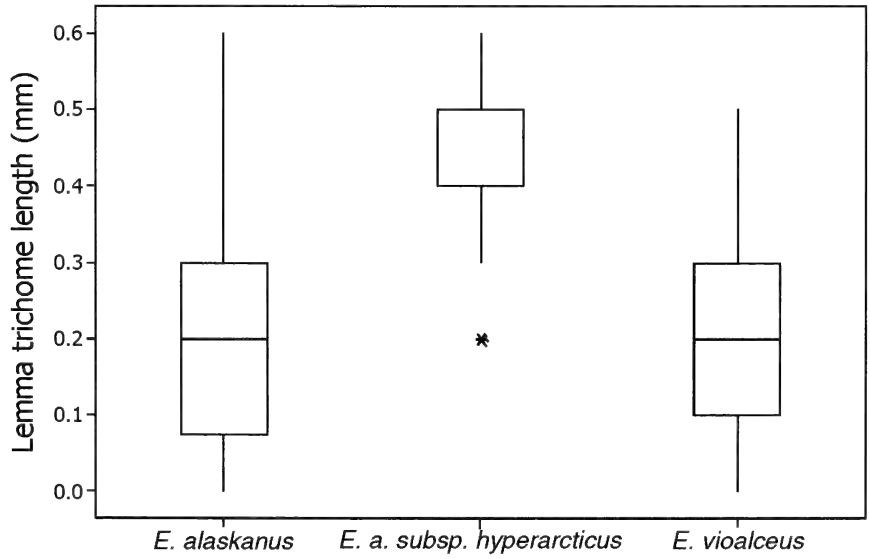


FIG. 3. Lemma trichome for *Elymus alaskanus sensu stricto* (n = 110), *E. alaskanus* subsp. *hyperarcticus* (n = 18) and *E. violaceus* (n = 169).

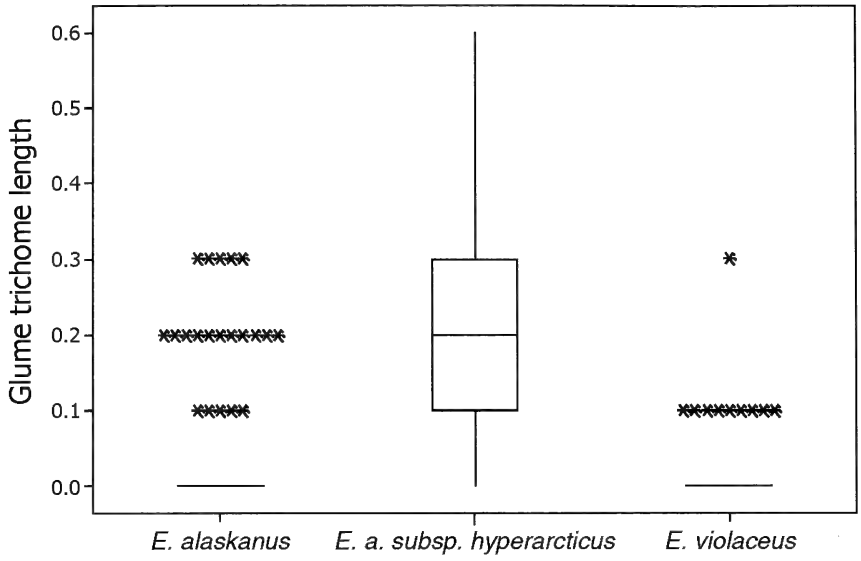


FIG. 4. Glume trichome length for *Elymus alaskanus sensu stricto* (n = 110), *E. alaskanus* subsp. *hyperarcticus* (n = 18) and *E. violaceus* (n = 169).

E. alaskanus or *E. alaskanus* subsp. *hyperarcticus* (Table 6). Pairwise comparisons among taxa of PCA factor 1 (Tukey 95% simultaneous confidence intervals) showed no significant differences between *E. alaskanus s.s.* and *E. alaskanus* subsp. *hyperarcticus*. However, *E. alaskanus* subsp. *hyperarcticus* was different from *E. violaceus*, and *E. alaskanus s.s.* was different from *E. violaceus*. ANOVA of PC2 scores showed highly significant differences among taxa (ANOVA, $F_{(2,283)} = 28.65$, $P < 0.001$; $R^2 = 0.136$), with *E. alaskanus* subsp. *hyperarcticus* different from both *E. violaceus* and *E. alaskanus s.s.* ANOVA of PC3 scores also confirmed highly significant differences among taxa (ANOVA, $F_{(2,283)} = 26.45$, $P < 0.001$; $R^2 = 0.151$). Pairwise comparisons among taxa of PC3 indicate significant differences among all taxa.

Discriminant analysis of morphological characters (Table 3) indicated that *E. alaskanus s.s.*, *E. alaskanus* subsp. *hyperarcticus* and *E. violaceus* were assigned to their true group 72.1%, 100% and 93.9% of the time, respectively. When using a subset of the total morphological characters, those characters used in the FNA (Barkworth et al. 2007) including glume to lemma ratio, glume trichome length and lemma trichome length, *E. alaskanus s.s.*, *E. alaskanus* subsp. *hyperarcticus* and *E. violaceus* were assigned to their true group 39.4%, 94.4% and 86.6% of the time, respectively. Cluster analysis results indicate that our observations could not be segregated into three discrete groups. All specimens fell within a single cluster.

Biogeographic analysis. Elevation differed among taxa when specimens were combined from all latitudes (Kruskall-Wallis, df = 2, $P < 0.001$ adjusted for ties; Fig. 6). However, significant differences for elevation between *E. alaskanus s.l.* and *E. violaceus* were not detected when specimens were grouped by latitude (1) below 55°N (Kruskall-Wallis, df = 1, $P < 0.090$ (adjusted for ties) (2) 55°N–60°N (Kruskall-Wallis, df = 1, $P < 0.0191$ (adjusted for ties) (3) above 60°N (ANOVA, $F_{(2,41)} = 0.09$, $P < 0.916$; $R^2 < 0.01$ adjusted). Note that there are no herbarium specimens of *E. alaskanus* subsp. *hyperarcticus* south of 60°N. Further, no evidence exists for association between taxa and habitat type (Fig. 7; Chi-square test $P < 0.528$). With the inclusion of recently collected specimens the distribution of the two species overlaps broadly, particularly in British Columbia (Fig. 8). This pattern differs markedly from data of Barkworth et al. (2007) where *E. alaskanus s.l.* was restricted to extreme northern BC and northward.

DISCUSSION

The close morphological association among taxa makes it difficult to differentiate among entities. We found, as Barkworth et al. (2007) did, that the glume to lemma ratio of *E. alaskanus s.s.* is significantly less than that of *E. violaceus*. Our average ratios indicate that the glumes of *E. alaskanus s.s.* and *E. alaskanus* subsp. *hyperarcticus* are on average ⅓ to ⅔ as long as the adjacent lemmas, and those of *E. violaceus* are ¾ to equal the lower lemma length (Fig. 1). Though the mean values for glume to lemma ratio concur with Barkworth et al. (2007), boxplots (Fig. 1) demonstrate that the range of overlap is too large for discrimination between the proposed species based on this character alone. Moreover, a subset

TABLE 5. COEFFICIENTS AND EIGENVALUES FOR THE FIRST THREE COMPONENTS OF *ELYMUS ALASKANUS SENSU STRICTO, E. ALASKANUS* SUBSP. *HYPERARCTICUS* AND *E. VIOLACEUS* INDIVIDUALS. * Percent of the total variability accounted for by each principle component.

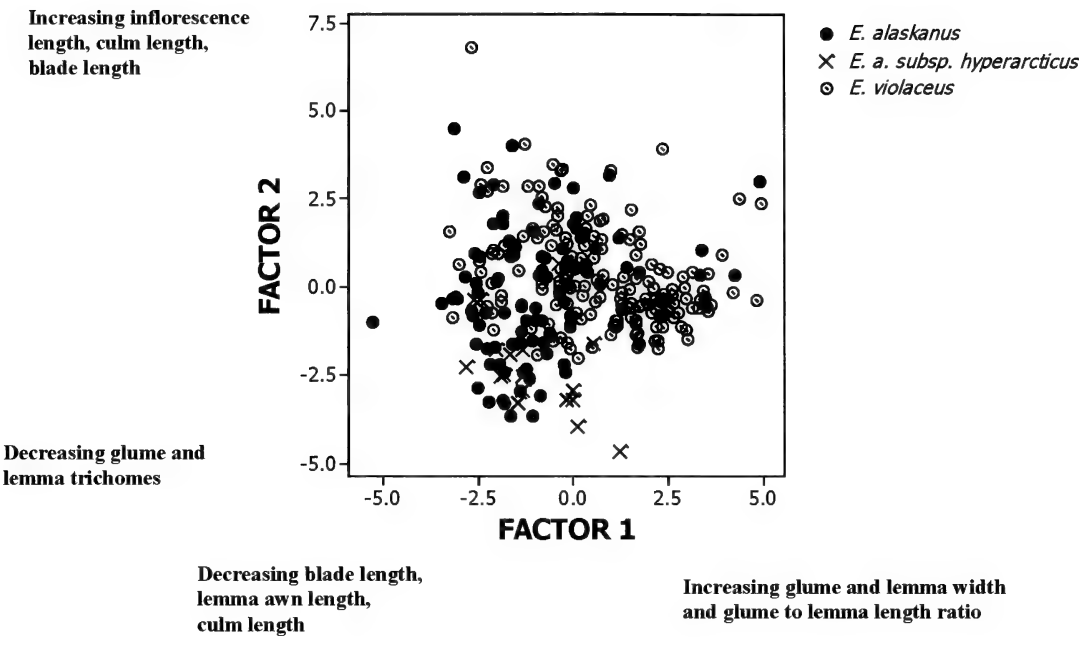
Variable	PC1 (20%)*	PC2 (15.7%)*	PC3 (11.1%)*
Culm length (cm)	−0.212	0.379	0.032
Blade width (cm)	−0.094	0.308	−0.279
Blade length (cm)	−0.236	0.344	−0.158
Inflorescence length (cm)	−0.178	0.424	−0.106
Inflorescence width (cm)	0.237	0.101	−0.273
Spikelet width (cm)	0.263	0.072	−0.378
Lower glume width (cm)	0.421	0.089	0.014
Lower glume awn length (mm)	0.099	0.085	−0.129
Number of glume veins	0.084	0.278	−0.014
Width of widest point of glume margin (mm)	0.296	0.041	−0.047
Lower lemma width (mm)	0.339	0.013	−0.323
Lower lemma awn length (mm)	−0.224	0.121	−0.247
Number of florets	0.085	0.248	−0.331
Lower glume trichome length (mm)	−0.115	−0.331	−0.335
Lower lemma trichome length (mm)	0.207	−0.289	−0.212
Lower glume to spikelet length ratio	0.318	0.139	0.336
Lower glume length to lower lemma length ratio	0.346	0.210	0.284
Width of widest point of glume margin to lower lemma length ratio	0.046	−0.147	−0.146

of specimens identified by Barkworth (Fig. 2) suggests that even when the distinguishing criteria are strictly applied, there is a continuum of values rather than discrete ranges for glume to lemma ratio that might indicate distinct entities. *Elymus alaskanus* subsp. *hyperarcticus* clearly has longer glume and lemma trichomes than the other taxa. *Elymus alaskanus s.s.* and *E. violaceus* trichome lengths are very similar (Figs. 3 and 4). These observations demonstrate that *E. alaskanus* subsp. *hyperarcticus* is easily distinguishable from other taxa as has been noted by others (Polunin 1940; Löve and Löve 1956; Hultén 1968; Welsh 1974; Tzvelev 1976; Löve 1984; Baum et al. 1991; Cody 1996; Barkworth 1997; Barkworth et al. 2007). Type specimens of the taxa were distinguishable based on lower glume to lower lemma ratio and followed the criteria outlined in the FNA (Barkworth et al. 2007). We expected the type specimens to fit the criteria outlined in the FNA (Barkworth et al. 2007) because they were named differently based on morphological differences of the particular specimens collected. However, it must be recognized that the usefulness of a type specimens for clarifying taxonomic issues may be limited because it represents only one population. Type specimens of *E. alaskanus* subsp. *alaskanus* (=*Agropyron alaskanum*), *E. alaskanus* subsp. *hyperarcticus* (=*Agropyron violaceum* var. *hyperarcticum*) and *Elymus violaceus* (=*Agropyron violaceum* var. *latiglume*) originated from Alaska, Nunavut and Montana, respectively and thus may be discrete compared to geographically intermediate material from British Columbia.

Using multivariate techniques we were unable to find a combination of characters that permit an unambiguous determination of groups at the specific level. Scatterplots of PCA factors 1–3 (Fig. 5) reveal a great deal of overlap among taxa, and the most defined group appears to be *E. alaskanus* subsp. *hyperarcticus*. Correlations between PCA scores and original traits are relatively low in magnitude, thus indicating that the morphological characters represent a small proportion of the overall variability. Discriminant analysis indicated that *E. alaskanus* subsp. *hyperarcticus* and *E. violaceus* could be assigned to their predefined taxonomic groups most of the time, but that *E. alaskanus s.s.* was a less reliable grouping. Further, we did a second discriminant analysis using a subset of data (glume to lemma ratio, glume trichome length and lemma trichome length) and found that *E. alaskanus s.s.* was correctly classified only 39.4% of the time. This may indicate that people making identifications have an easier time classifying *E. violaceus* and *E. alaskanus* subsp. *hyperarcticus* specimens than they do *E. alaskanus s.s.* specimens, however why this might be remains unknown. We used cluster analysis to determine if specimens could be put into groups that were not defined in advance but the results indicate that the observations were not divisible into groups.

According to Barkworth et al. (2007) *E. alaskanus s.l.* is thought to inhabit lower elevations than *E. violaceus*. Our analysis indicates a trend for *E. violaceus* to be at higher elevations below 60°N, but these differences were not significant (Fig. 6). Above 60°N no differences were detected among taxa. Environmental conditions to which plants are exposed at similar elevations are not constant across latitudes (Pojar and MacKinnon 1994), and this may explain our

(a)



(b)

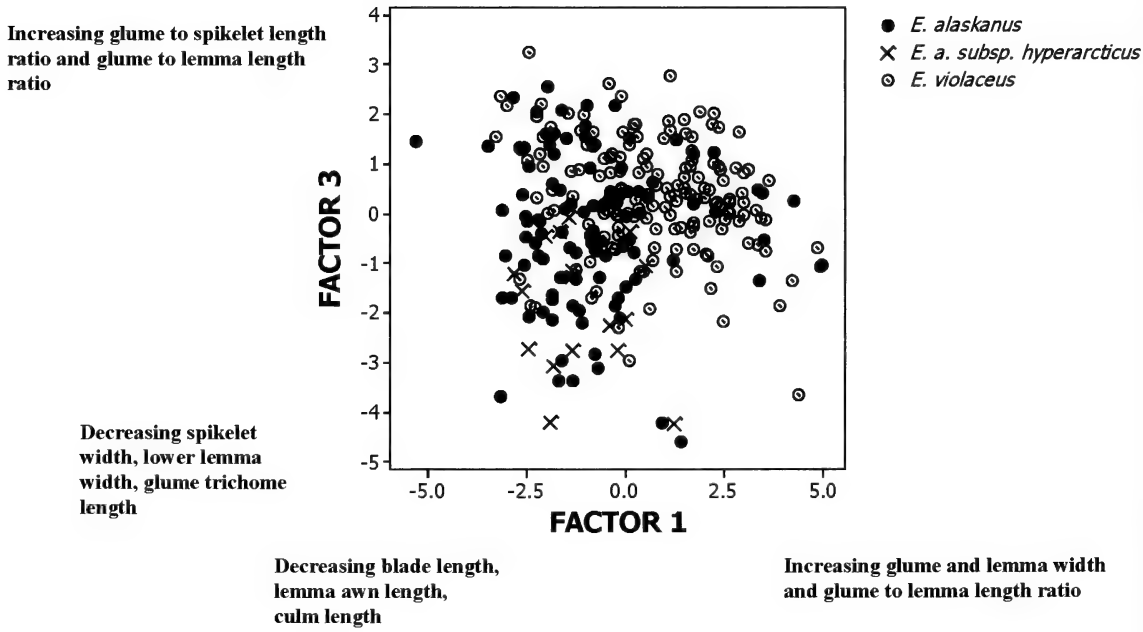


FIG. 5. Scatter graphs of principal components scores in pairwise relationships: a) factor 1 vs. factor 2; b) factor 1 vs. factor 3; c) factor 2 vs. factor 3. See Table 5 for the morphological characters included in the analysis.

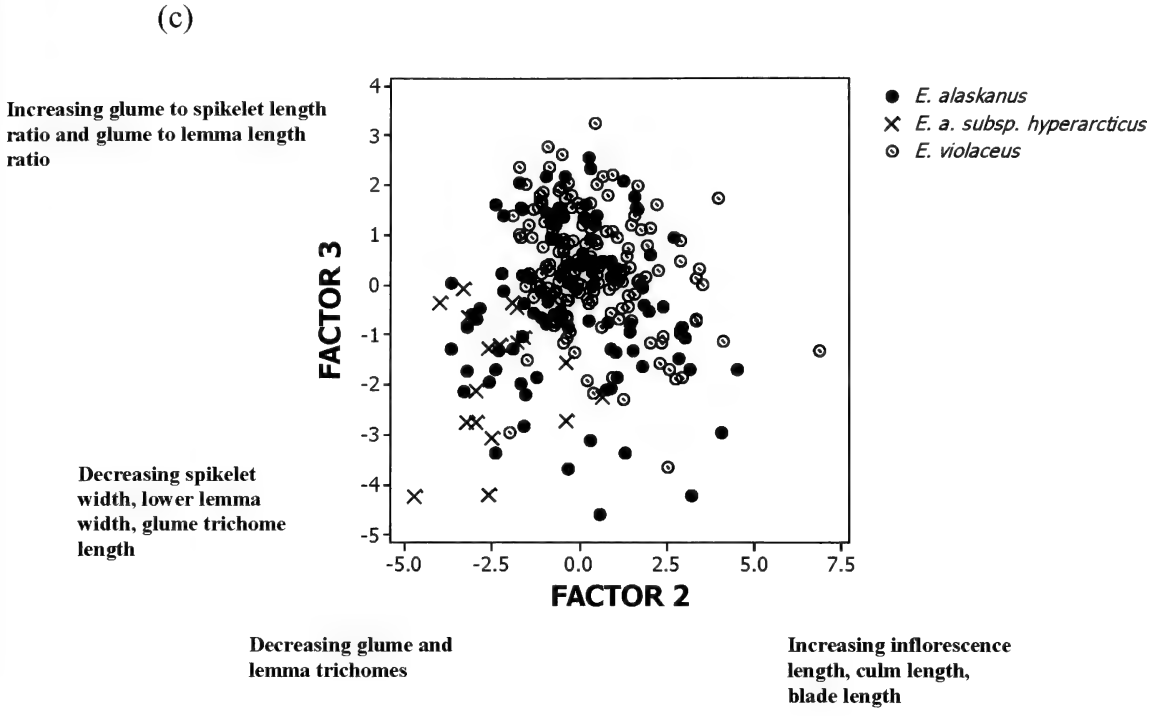


FIG. 5. Continued.

results. As a general rule, species occur at lower elevations as one moves north. At lower latitudes, plants inhabiting higher elevations are exposed to similar environmental conditions (e.g., extremes in daily temperature, shorter growing season, limited water supply, exposure to wind and colder temperatures) as plants at lower elevations but higher latitudes (Forbes 1997; Sohlberg and Bliss 1984). When latitude is not considered *E. violaceus* does appear to be found at higher elevations than *E. alaskanus s.l.* taxa which may explain the current perception that *E. violaceus* is found at higher elevation.

Contrary to Barkworth et al. (2007) who contend *E. alaskanus s.l.* is often associated with valleys/ flat areas and *E. violaceus* restricted to rocky habitats, we found that both *E. alaskanus s.l.* and *E. violaceus* were approximately equally likely to occur in either habitat type (Fig. 8). Based on our analysis, habitat cannot be used to differentiate among taxa. Habitat data recorded on herbarium sheets may be too general in order to make inferences about micro-habitat preferences. In order to analyze primary habitat difference future research should include a detailed and standardized procedure for scoring such habitat characteristics.

In the past, specimens of *E. alaskanus s.l.* have not been widely reported throughout British Columbia nor as far south as in our study (Barkworth et al. 2007). With the inclusion of new collections our map (Fig. 8) of *E. alaskanus s.l.* and *E. violaceus* demonstrates that the distributions of the two taxa overlap broadly in range, particularly in British Columbia south of 60°N, except on the coast where no *E. alaskanus s.l.* occurs. *E. alaskanus* subsp. *hyperarcticus* only occurs north of 60°N. Biogeographically, the distributions of *E. alaskanus s.l.* and *E. violaceus* are of interest because it is surprising that such closely related species should both have spread and colonized similar and relatively isolated geographical areas, such as Greenland for example, since the last ice-age.

Nomenclatural Considerations

Deciding how different a taxon must be to warrant consideration as a separate entity has guided this study. In order to validate differentiating between species it is necessary to have a character or combination of characters that can discriminate unequivocally between them (Barkworth 1992). According to Barkworth et al. (2007) infraspecific taxa that show clear morphological and ecological distinctions are treated as subspecies. Despite a large sample size, wide geographic breadth and inclusion of morphological characters currently used to discriminate between *E. alaskanus s.l.* and *E. violaceus* in the Flora of North America (Barkworth et al. 2007), no clear difference morphologically, geographically or in habitat could be established in our study. According to taxonomic ranking rules

TABLE 6. ANOVA RESULTS OF PC1–3 VERSUS TAXON (*ELYMUS ALASKANUS SENSU STRICTO, E. ALASKANUS* SUBSP. *HYPERARCTICUS* AND *E. VIOLACEUS*). PC1 (R^2 = 0.1625); PC2 (R^2 = 0.1359); PC3 (R^2 = 0.1516).

	Source	DF	SS	MS	F	P
PC1	TAXON	2	172.35	86.17	28.65	<0.001
	Error	283	851.22	3.01		
	Total	285	1023.57			
PC2	TAXON	2	114.21	57.10	23.41	<0.001
	Error	283	690.32	2.44		
	Total	285	804.53			
PC3	TAXON	2	89.34	44.67	26.45	<0.001
	Error	283	477.87	1.69		
	Total	285	567.20			

following the International Code of Botanical Nomenclature a subspecies should be more similar to its parent species than different species are to one another (McNeill et al. 2006). Yet, the most distinct entity in the group studied here was *E. alaskanus* subsp. *hyperarcticus*. In fact Barkworth (1997), after examining specimens of *E. alaskanus* subsp. *hyperarcticus*, suggests that the entity is so distinct that it should not be included in the same species as *E. alaskanus* subsp. *alaskanus* and recommended it be group within *E. sajanensis* (Nevski) Tzvelev as Tzvelev (1976) had done (Fig. 1). If morphological differences between *E. alaskanus s.s.* and *E. alaskanus* subsp. *hyperarcticus* warrant subspecies designation than how could less variation between *E. alaskanus s.s.* and *E. violaceus* warrant species designation?

Preparing morphological identification keys when the characters holding a group together are non-morphological is not practical. Based on this study, there is no meaningful method to separate North American *E. alaskanus s.s.* and *E. violaceus* either morphologically or geographically. Thus, we propose a nomenclatural reconsideration of the *E. alaskanus s.s.* and *E. violaceus* complex based on the specimens used in this study and suggest that *Elymus alaskanus* is most correctly applied to all specimens that we examined following the International Code of Botanical Nomenclature (McNeill et al. 2006). Concurrent with the treatments of Löve (1984), Cody (1996) and Barkworth et al. (2007), *E. alaskanus* subsp. *hyperarcticus* should continue to be treated as a subspecies of *E. alaskanus*. Subspecific recognition is warranted for *E. alaskanus* subsp. *hyperarcticus* based on glume and lemma trichome length. With respect to this feature, Barkworth et al. (2007) consider the trichomes of *E. alaskanus* subsp. *alaskanus* up to 0.2mm long and *E. alaskanus* subsp. *hyperarcticus* trichomes 0.2–0.5mm long. We observed that some trichomes of *E. alaskanus* subsp. *alaskanus* could reach 0.3mm rather than 0.2mm and some trichomes of *E. alaskanus* subsp. *hyperarcticus* could reach 0.6mm. Also, glume trichomes exceeded the glume margins in every specimen of *E. alaskanus* subsp. *hyperarcticus*. In the future, an analysis in which trichome density is quantitatively assessed may be useful.

We recommend the name *E. alaskanus* subsp. *alaskanus* continue to be used for those specimens with glabrous glumes or glumes covered sparsely by trichomes following Barkworth et al. (2007). Unlike the treatment in the Flora of North America (Barkworth et al. 2007), we believe *E. violaceus* should not be regarded as a separate species from *E. alaskanus* for those specimens with relatively long glumes. If recognized at all, it should be considered a subspecies of *E. alaskanus*. At the sub-specific level, the epithet "*latiglumis*" has priority following Article 11.4 of the International Code of Botanical Nomenclature (McNeill et al. 2006). The most appropriate name for those entities with relatively long glumes is *E. alaskanus* subsp. *latiglumis* rather than *E. violaceus* which would be the name that takes priority at the specific level. It would be practical to follow the treatment of Barkworth et al. (2007) and call specimens with glumes 1/3–2/3 as long as the adjacent lemmas *E. alaskanus* subsp. *alaskanus* or *E. alaskanus* subsp. *hyperarcticus* (depending on trichome length) and specimens with glumes 3/4 as long as, to slightly longer than the adjacent lemmas, *E. alaskanus* subsp. *latiglumis*. Based on our observations, there is no evidence for a third taxon in the complex, namely *E. violaceus*, within the region of our study. Having not compared *E. violaceus* specimens used in this study to Scandinavian and Greenlandic specimens we cannot comment on whether or not they are similar entities to those found in British Columbia. For a thorough taxonomic revision of the complex, field and population studies over the whole circumboreal distribution must be made. Common garden experiments would be useful to examine specific morphological character differences as well.

This study illustrates the challenges to taxonomists of creating effective dichotomous keys that reflect biological reality. We attempted to differentiate between *E. alaskanus* and *E. viola-*

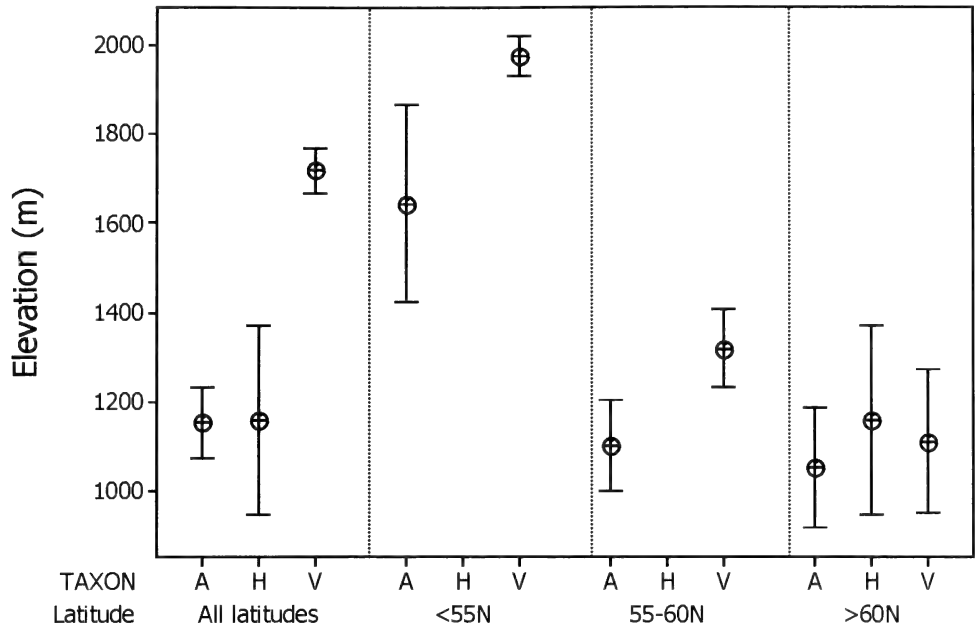


FIG. 6. Mean elevation (m) of taxa for 4 categories of latitude: (1) all latitudes (A: n = 54, H: n = 7, V: n = 128); (2) <55°N (A: n = 7, V: n = 83); (3) 55°N–60°N (A: n = 26, V: n = 29); (4) >60°N (A: n = 21, H: n = 7, V: n = 16). Bars are one standard error from the mean. *E. alaskanus* (A); *E. alaskanus* subsp. *hyperarcticus* (H); *E. violaceus* (V).

ceus using published diagnostic features but were unable to do so using morphological characters, habitat preferences, or geographic distribution. We determined that the range of overlap of significant morphological characters examined of *E. alaskanus* and *E. violaceus* was too great to discriminate between taxa. We also found that *E. violaceus* and *E. alaskanus* inhabit similar habitats and have overlapping geographic ranges and elevations. Our analysis indicates that *E. alaska-*

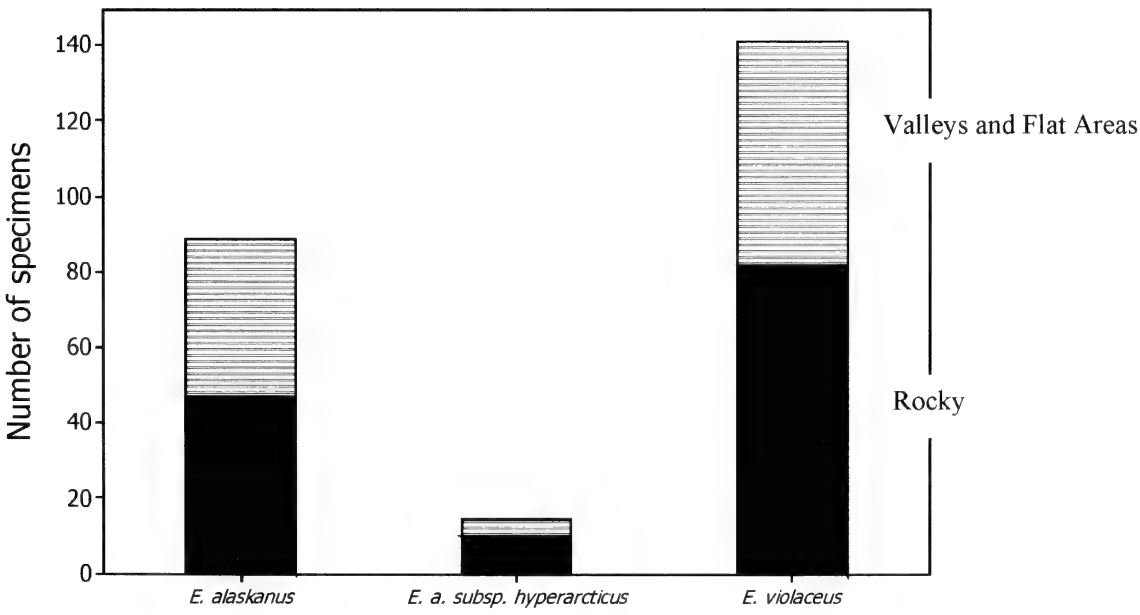


FIG. 7. A mosaic plot for habitat type and taxa. The stripped bars represent the number of specimens found in valleys and flat areas and the black bars represent the number of specimens found in rocky habitats. *E alaskanus sensu stricto* n = 89; *E. alaskanus* subsp. *hyperarcticus* n = 15; *E. violaceus* n = 141.

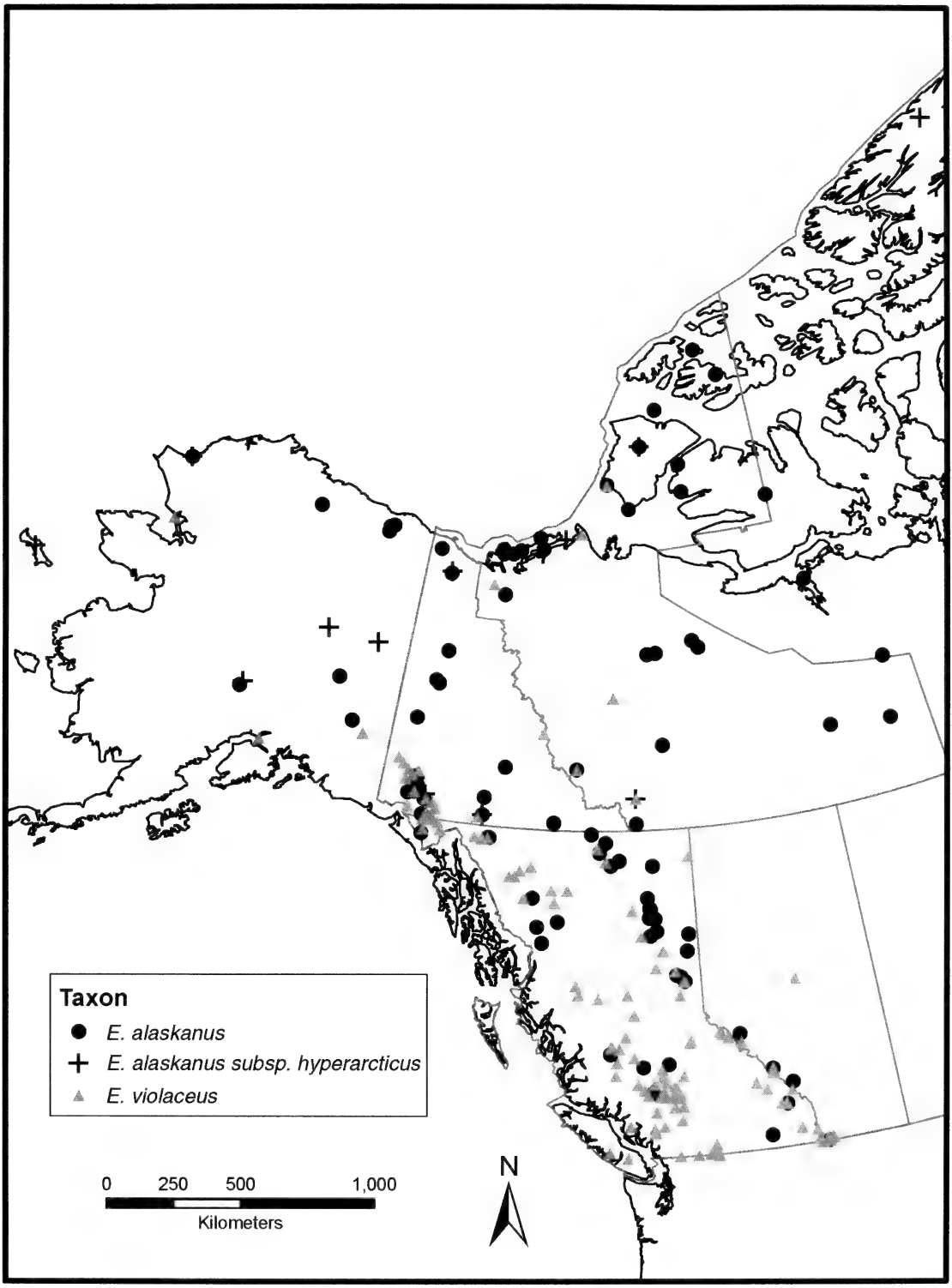


FIG. 8. Geographic distribution of *Elymus alaskanus sensu stricto*, *E. alaskanus* subsp. *hyperarcticus* and *E. violaceus* specimens from Alaska, Alberta, British Columbia, Northwest Territories, and Yukon used in this study.

nus and *E. violaceus* are potentially the same species with three infraspecific subspecies including *E. alaskanus* subsp. *alaskanus*, *E. alaskanus* subsp. *latiglumis* and *E. alaskanus* subsp. *hyperarcticus*. New geographic distribution records of specimens, particularly in British Columbia, should be included in future maps of the species ranges. For future analysis we recommend a similar analysis of other closely related species such as *E. scribneri* (Vasey) M.E. Jones and *E. trachycaulus* (Link) Gould with which *E. violaceus* has been known to form intermediates and *E. macrourus* (Turcz.) Tzvelev of which large specimens of *E. alaskanus* resemble (Barkworth et al. 2007). Further morphological analysis in combination with genetic studies including the European and eastern North American part of range may help clarify relationships between taxa. Knowledge concerning genetic relationships among these taxa is still incomplete, but the accumulation of information suggests a close genetic relationship between E. *alaskanus* and *E. violaceus,* thus supporting our findings (Zhang et al. 2000, 2002; Sun and Salomon 2003; Sun et al. 2006). Using morphological types based on spike and vegetative characters, Zhang et al. (2000) investigated genetic variation and structure among *Elymus alaskanus* populations from a broad geographical area and found that allozyme patterns revealed clear similarities among types of "tall *hyperarcticus*", "*hyperarcticus*", "*latiglumis*", "*virescens*", and "*violaceus*". The taxon "*violaceus*" was found to be more similar to "*hyperarcticus*" and "*latiglumis*" then to "*virescens*" (Zhang et al. 2000). Zhang et al (2002) and Sun and Salomon (2003) report that morphological types "*violaceus*" and "*latiglumis*" are genetically more similar to each other than to "*hyperarcticus*", though later Sun et al. (2006) found a close genetic relationship between *E. alaskanus* subsp. *hyperartcicus* and *E. violaceus*. Future genetic studies should clarify how differentiation among morphological types was made, particularly between "*violaceus*" and "*latiglumis*" types given that these are currently regarded as synonyms (Stewart and Barkworth et al. 2001; Soreng et al. 2003; Barkworth et al. 2007). Studies which correlate morphology with genetic variability may help clarify the relationships between taxa.

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APPENDIX 1

SPECIMENS EXAMINED

* = Accessions included in mapping analysis. ° = Accessions included in habitat analysis. Herbarium abbreviations: **V** = Royal BC Museum; **CAN** = Canadian Museum of Nature; **UBC** = University of British Columbia; **US** = United States National Herbarium.

***Elymus alaskanus* (Scribn. & Merr.) Á. Löve**—CANADA. ALBERTA. **UBC**: 62034*°, 82554*. BRITISH COLUMBIA. **V**: 61973*, 76995*°; 105917*°, 106136*°, 125951*, 16671*, 17803*, 24489*, 79126*°, 91608*°, 194719*°, 195414*°, 195468*°, 196196*°, 196201*°, 196244*°, 196245*°, 196433*°, 198508*°, 198528*°, 198554*°, 198634*°, 198638*°, 198656*°, 198740*°, 198752*°, 198759*°, 198762*°, 198879*°,

198883*°, 198895*°, 198926*°, 198931*°, 198961*°, 199623*, 199783*°; **UBC**: 169655*°, 42328*°. NORTHWEST TERRITORIES. **CAN**: 127440*°, 127441*°, 127442*°, 127443*°, 127444*, 200030*°, 203081*°, 203082*°, 203084*, 268362*°, 270867*, 279113*, 279114A*°, 279322*°, 39283*°, 39286*°, 39288*, 39329*, 527868*°, 529498*, 530883*°, 530891*°, 582469*°, 584015*°, 585091*°, 585093*°; **UBC**: 111282*°, 113135*, 113185*, 171348*°, 171489*°, 171504*°, 171572*°, 36871*, 37095*°, 90155*°; V25042*°. QUEBEC. **V:** 114219. YUKON. **CAN:** 276347*°, 276351*°, 276598*°, 303292*°, 306804*°, 318450*, 39772*°, 454931*°, 53085*°, 549414D*°; **UBC:** 119413*°, 181579°, 27873*°, 99014*°, 99023*°, 99743*°; **V**: 118217*°, 118228*°, 122789*, 137591*°, 137592*, 137610*°, 137611*°. USA. ALASKA. **CAN**: 211188*°, 211190*°, 211191*°, 248032*°, 274084*°, 211188*°, 211190*°, 211191*°, 248032*°, 274084*°, 276349*°, 367095*°, 514133°, 514134*°; **US**: 592341 holotype.

***Elymus alaskanus* subsp. *hyperarcticus* (Polunin) Á. Löve & D. Löve**—CANADA. NORTHWEST TERRITORIES. **CAN:** 203083*, 203085*°, 225486°, 279114B*°. NUNAVUT. **UBC:** 184460*°; **US:** 203113 isotype. YUKON. **CAN:** 260928*°, 270276*°, 454932*°; **UBC:** 99024*, 115538*°, **V:** 198867*°. USA. ALASKA. **CAN:** 225257*°, 270277*°, 274083*°, 318764*°, 366745*°, 367096*°; **V:** 37905*.

***Elymus violaceus* (Hornem.) J. Feilberg**—CANADA. ALBERTA. **CAN:** 514030*; **UBC:** 21928*°, 77875*°; **V:** 25062*°. BRITISH COLUMBIA. **UBC:** 145869*°, 145871*°, 145872*°, 155889*°, 155890*°, 156195*°, 17254*, 17375*, 17410*°, 17413*, 17429*, 21923*°, 21925*°, 220654*°, 45622*°, 58312*, 60491*°, 67864*°, 86401*°, 86433*°, 98384*°, 988386*°; **V:** 123194, 104896*°, 106180*°, 106188*°, 107666*°, 112825*°, 11309*°, 115058*°, 118641*°, 118669*°, 118989*°, 119525*°, 119606*°, 119616*°, 119758*°, 119767*°, 120201*°, 120270*°, 120310*°, 127184*°, 127185*, 127186*°, 127187*, 131360*°, 132206*°, 137599°, 13699*, 137663*°, 141176*°, 141179*°, 147702*°, 147703*, 147705*°, 148290*°, 160614*°, 160623*, 163871*, 16741*, 170331*°, 17763*, 184000*°, 188109*°, 18826*, 189980*°, 189981*°, 191286*°, 191307*°, 191896*°, 196248*°, 199824*°, 200057*°, 200534*°, 200900*°, 200910*°, 200979*°, 201806*°, 23978*°, 25520*°, 27856*°, 27867*°, 30232*°, 31833*°, 32552*°, 36900*°, 36919*°, 36929*°, 36943*°, 404*, 44524*°, 44565*°, 48251*°, 58714*°, 59089*°, 61972*, 69404*°, 71451*°, 71457*°, 75509*°, 76343*°, 76927*°, 7695*, 79578*°, 80869*°, 83134*°, 83135*°,83137*°, 83139*°, 83171*°, 83172*°, 83780*°, 87478*°, 87478*°, 87482*°, 88408*°, 88434*°, 88444*°, 91014*°, 91060*°, 91279*°, 91346*°, 91374*°, 91562*°, 91576*°, 91865*°, 91878*°, 92000*°, 92641*,93241*°, 96089*°, 96733*°, HR08020*, 117436*°. NORTHWEST TERRITORIES. **CAN:** 39289*; **UBC:** 182645*, 18398*, 83427*, 90154*°, 96157*°; **V:** 141141*°, 141142*°. ONTARIO. **UBC:** 17437. USA. ALASKA. **CAN:** 514025*°, 514027*°, 514028*°. MONTANA. **V:** 44690; **US:** 556692 holotype. UTAH. **V:** 141282°. WASHINGTON. **V**: 96357°, 137603°. YUKON. **UBC**: 99022*°, 99658*°; **V**: 137595*°, 137604*°, 137605*, 137607*°, 137608*°, 137609*°, 137612*°, 137613*°, 87601*°, 87657*°, 87738*°, 87857*°, 98891*°.

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CHROMOSOME COUNTS AND TAXONOMY OF *MENTZELIA THOMPSONII* (LOASACEAE)

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ABSTRACT

The species *Mentzelia thompsonii* Glad was published in 1976 based solely on herbarium material, and it is the only species in *Mentzelia* section *Trachyphytum* that has not been examined cytogenetically. Here we report that *M. thompsonii* is diploid ($2n = 18$), making it the easternmost diploid species in section *Trachyphytum* and the only one that does not occur in California. The diploid status and edaphic specialization of *M. thompsonii* suggest that it is a paleoendemic isolated from other diploids in *Trachyphytum* by specialization during Pleistocene climate change. Our investigations have also uncovered confusion in the literature and herbaria regarding the taxonomy of *M. thompsonii* and its overall place within *Mentzelia*. *Mentzelia thompsonii* has been synonymized with *Mentzelia humilis* (Urb. & Gilg) J. Darl. (a member of section *Bartonia*) in several prominent databases and herbaria. To the contrary, our studies reveal that *M. thompsonii* is distinct from *M. humilis*; furthermore, as a unique component of Colorado Plateau diversity, it is critical for inferences of biogeographic evolution in section *Trachyphytum*.

Key Words: *Acrolasia humilis*, biogeography, diploid, Mancos Shale, *Mentzelia humilis*, *Mentzelia thompsonii*, Pleistocene climate change, polyploidy.

Mentzelia thompsonii Glad (Fig. 1) is a small annual confined to the Colorado Plateau of the southwestern United States. Its range extends from the Four Corners region north along the Utah-Colorado border to the Uinta Basin (Fig. 2). The species is an edaphic endemic, usually occurring on barren, salty soils derived from the Mancos Shale Formation (Glad 1976; Holmgren et al. 2005; Brokaw 2009). *Mentzelia thompsonii* is one of the most recently described species in section *Trachyphytum* (Glad 1976), and its evolutionary significance within this group is only now becoming apparent (Brokaw and Hufford 2010a, b). Prior to cytogenetic surveys of the group, only eight North American species were recognized in section *Trachyphytum* (Loasaceae) (Darlington 1934). However, the discovery of extensive polyploidy (Zavortink 1966) and coincident reproductive barriers subsequently led to the recognition of over 20 species in the southwestern United States alone. *Mentzelia thompsonii* was described ten years after Zavortink's (1966) biosystematic revision of *Trachyphytum*, and it is the only North American species currently lacking a chromosome count. Recent work has suggested that section *Trachyphytum* represents a monophyletic group exhibiting complicated polyploid evolution (Hufford et al. 2003; Brokaw and Hufford 2010a, b), and verification of the ploidal level of *M. thompsonii* is vital to the interpretation of molecular evolution and gene flow in the group.

The goals of the study were: 1) to collect chromosome data for *M. thompsonii* and 2) to develop a set of chromosomally vouchered molecular and morphological samples for systematic analyses. While these cytogenetic analyses have been essential to determine *M. thompsonii*'s role in the polyploid complexes of section *Trachyphytym*, our investigations have also led to new insights regarding the biogeography of *Trachyphytum* and resolved confusion in the literature and herbaria regarding the taxonomy and validity of *M. thompsonii*.

MATERIALS AND METHODS

Populations examined in this study were selected to represent the northwestern (Uinta Co., UT, *Brokaw 234*) and southeastern (San Juan Co., NM, *Brokaw 345*) limits of the

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FIG. 1. Habit of *Mentzelia thompsonii* Glad. Photograph courtesy of W. A. Weber (University of Colorado).

distribution of *M. thompsonii*. Chromosome counts were made from field-collected microsporocytes fixed in Farmer's solution (3 parts 95% ethanol: 1 part glacial acetic acid) at the time of voucher collection. Following the procedures outlined by Windham (2000), fixed materials were stored at −20°C and transferred to 70% ethanol immediately before making slides. Dissected anthers were macerated in a drop of 1% acetocarmine stain, which was mixed 1:1 with Hoyer's solution prior to setting the cover slip and squashing. Slides were examined with an Olympus BH-2 phase contrast microscope, and representative cells were photographed using Kodak Technical Pan 2415 film. The voucher specimens, *Brokaw 234* (WS375612) and *Brokaw 345* (WS375773), have been deposited at the Marion Ownbey Herbarium (WS). Additional duplicate vouchers have been sent to ACU and COLO.

RESULTS AND DISCUSSION

Analyses of microsporocytes undergoing meiosis revealed that the chromosome number of both the northwestern and southeastern populations of *M. thompsonii* is $n = 9$. The chromosomes consistently formed nine bivalents during the reductional division (Fig. 3a) and these segregated normally during anaphase II to produce four daughter cells containing nine chromatids each (Fig. 3b). The base chromosome number of *Mentzelia* section *Trachyphytum* is $x = 9$, making *M. thompsonii* a diploid. It is the easternmost diploid in the section and the only one that does not occur in California (Fig. 2). Further, it is the only diploid species whose current distribution does not overlap with any other diploid in *Trachyphytum*.

Evolutionary Ecology

The discovery that *Mentzelia thompsonii* is diploid has important implications for our understanding of biogeography and evolution in section *Trachyphytum*. The lineage of *M. thompsonii* is nested within the section *Trachyphytum* clade (Brokaw and Hufford 2010a) leading to the most parsimonious hypothesis that *M. thompsonii* represents a range extension far from the California origin of the *Trachyphytum* diploids. The section has its greatest species richness and representatives of all its major clades in southern California. The polyploid taxa generally have larger distributions, extending further north and east than those of diploids (Zavortink 1966). Prior to our investigation of *M. thompsonii*, only polyploid taxa were known to have gotten as far east as the Colorado Plateau. Given that polyploids are derived from diploids and thus more recently evolved, analyses of *Trachyphytum* lacking *M. thompsonii* would suggest that range expansions were associated novel trait combinations acquired during or following polyploidization. However, the geography of the diploid *M. thompsonii* represents a major exception to this generalization, suggesting that other factors must be considered.

Patterns of edaphic specialization in *Trachyphytum* may partly explain the disjunct range of *M. thompsonii*. Only two other species in *Trachyphytum*, the tetraploid *M. mollis* M. Peck and the octoploid *M. packardiae* Glad, occur entirely outside California; both are limited to unusual soils (Glad 1975, 1976). Although soils were not available to her for chemical analyses, Glad (1976) first noted that shales and grey clays of the Mancos Formation were commonly listed as substrates on specimen labels for *M. thompsonii*. With a wider sampling of populations, it is now evident that *M. thompsonii* is limited almost exclusively to the Mancos Shale and other Cretaceous marine sediments of the Colorado Plateau (Holmgren et al. 2005; Brokaw 2009). Thus, all three *Trachyphytum* species absent from California appear to be associated with substrate specialization.

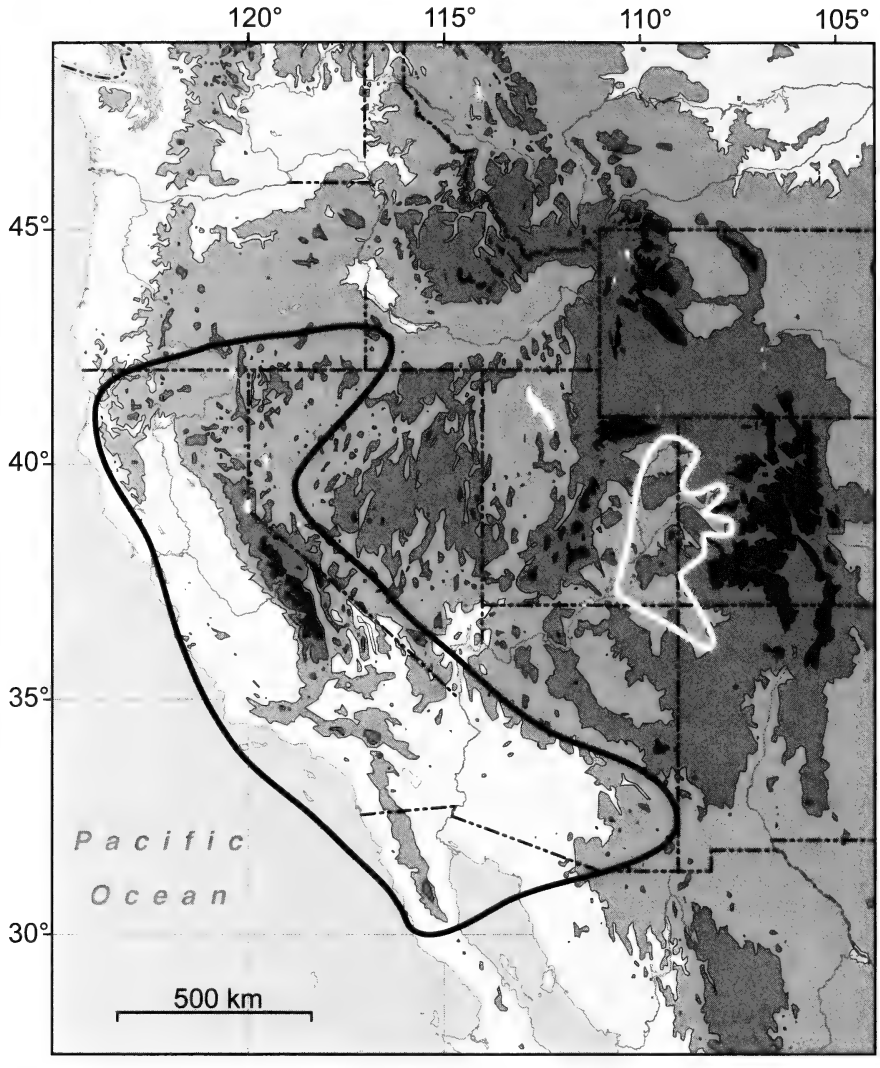

FIG. 2. Range of *Mentzelia thompsonii* in eastern Utah, western Colorado, and Four Corners region (white) and combined ranges of all other diploid species in *Mentzelia* section *Trachyphytum* in California, southern Oregon, southwestern Idaho, western Nevada, southwestern Arizona, and northwestern Mexico (black).

These observations suggest that the simplest biogeographic hypothesis (a one-way expansion from California) may be insufficient to fully explain species distributions in *Trachyphytum*. The range of *M. thompsonii* shows that diploids have accomplished substantial range expansion outside of California. The current abundance of polyploids in intervening regions of the Great Basin and western Colorado Plateau suggest that a similar distribution of ancestral diploids is at least plausible. It is possible that diploid populations formerly in this region have been displaced by competition. This line of reasoning, coupled with observed edaphic specialization, suggests that *M. thompsonii* may be a paleoendemic, i.e., a species isolated through extinctions of close relatives (Stebbins and Major 1965). Major migrations and extinctions of diploid populations could have been driven by dramatic shifts in vegetation during Pleistocene climate change (Dynesius and Jansson 2000; Thompson and Anderson 2000; Minnich 2007). During vegetational shifts, the ancestors of *M. thompsonii* may have persisted in northeastern portions of the diploid ranges by specializing for edaphically stressful habitats where most competing vegetation was excluded.

It is likely that shifting diploid ranges during the Pleistocene facilitated hybridization, leading to the extensive generation of allopolyploids in *Trachyphytum* documented by Brokaw and Hufford (2010b). *Mentzelia thompsonii* is one of the few diploids in *Trachyphytum* lacking allopolyploid descendents (Brokaw and Hufford 2010b), which is not surprising considering its current isolation from other extant diploids. The

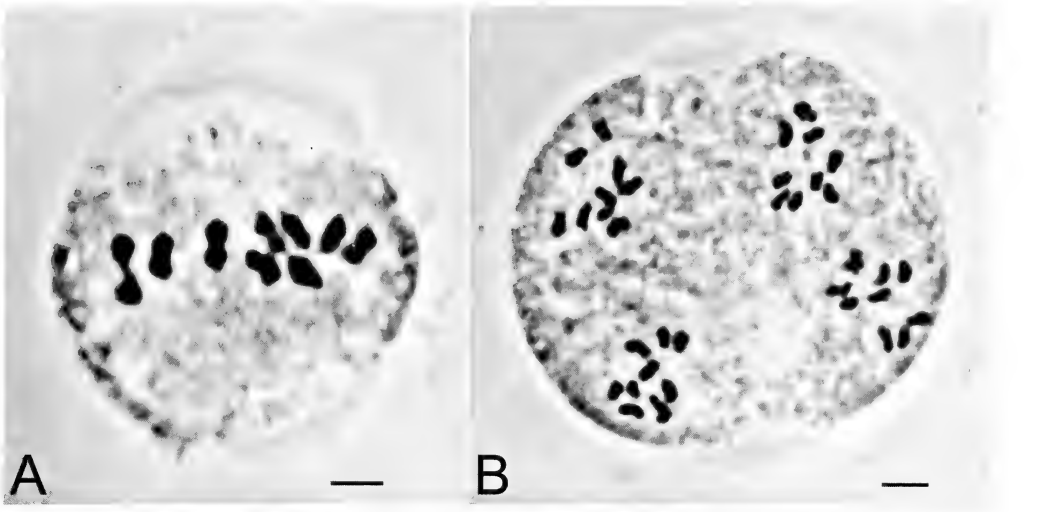

FIG. 3. Chromosomes of *Mentzelia thompsonii* (*Brokaw 234*) at: A) Metaphase I cell showing nine pairs of chromosomes; B) Anaphase II cell showing four daughter nuclei each containing nine chromatids. Scale bars = 5 μm.

species almost certainly had fewer opportunities than most diploids for allopolyploid hybridizations in Pleistocene ice age refugia. However, molecular data have implicated *M. thompsonii* in one introgressional event without polyploidization (Brokaw and Hufford 2010a, b). Brokaw and Hufford (2010a) showed evidence of recombination between nuclear genes of *M. thompsonii* and the Sonoran Desert diploid *M. affinis* Greene, suggesting gene flow between these species. Although *M. thompsonii* and *M. affinis* are not currently sympatric, populations of *M. affinis* approach the range of *M. thompsonii* more closely than those of any other diploid in *Trachyphytum*. *Mentzelia affinis* occurs in southern and western Arizona, separated from the nearest *M. thompsonii* populations (in the Four Corners region) by less than 400 km. The suitability of the intervening habitat during the Pleistocene is unclear, but it is interesting to note that a similar geographic pattern has been observed in the genus *Boechera* (Brassicaceae). In this instance, microsatellite data (Windham et al. unpublished) reveal that *B. perennans* (S. Watson) W. A. Weber (with a southern Arizona range similar to *M. affinis*) has hybridized with *B. pallidifolia* (Rollins) W. A. Weber, a Colorado Plateau endemic that reaches its southern limit near the Four Corners (like *M. thompsonii*). The striking similarity of these two cases suggests that genetic interactions between species occupying the warm deserts of southern Arizona and the cool deserts of the Four Corners region may be more common than previously supposed.

Molecular data have revealed that unexpected interactions between species (like those inferred between *M. thompsonii* and *M. affinis*) are relatively common in *Mentzelia* section *Trachyphytum* (Brokaw and Hufford 2010a, b). These provide intriguing evidence that Pleistocene migrations have contributed to complicated patterns of molecular evolution, ecological specialization, and a burst of allopolyploid speciation (Brokaw and Hufford 2010a, b). The discovery that *M. thompsonii* is diploid adds another piece to the puzzle, allowing us to view our biogeographic hypotheses in a new light and critically examine the effects of geographic isolation on species evolution in *Mentzelia* section *Trachyphytum*.

Taxonomic Status

Mentzelia thompsonii is a poorly known taxon, rarely collected and confined to unusual substrates in a region of the United States that is only now receiving the botanical exploration it deserves (Heil et al. in press). This largely explains why it was overlooked by both previous monographic treatments of *Trachyphytum* (Darlington 1934; Zavortink 1966). Not only has this led to the delayed cytogenetic study of *M. thompsonii*, but it has also contributed to a complicated nomenclatural history and, ultimately, to the synonymization of the taxon in prominent databases and herbaria. In fact, *M. thompsonii* is still listed as "not accepted" by the Integrated Taxonomic Information System (ITIS 2011) and the PLANTS Database (USDA-NRCS 2011).

Through a series of nomenclatural and taxonomic errors, *M. thompsonii* (Fig. 4) has been incorrectly synonymized with *M. humilis* (Urb. & Gilg) J. Darl. (Fig. 5), a distantly related member of section *Bartonia*. The initial confusion in the taxonomy of *M. thompsonii* stems from a long-

FIG. 4. Holotype of *Mentzelia thompsonii* Glad.

running disagreement between authors who recognize *Trachyphytum* as a section of the genus *Mentzelia* (Torrey and Gray 1840; Urban and Gilg 1900; MacBride 1918; Darlington 1934; Thompson and Roberts 1974; Hufford et al. 2003) and authors who segregate the group as the genus *Acrolasia* (Presl 1831; Rydberg 1903; Davidson 1916; Weber and Wittman 2001). Following Rydberg's (1903) recircumscription of *Acrolasia*, the entity now known as *M. thompsonii* was first described and published as *Acrolasia humilis* by Osterhout in 1922. The epithet *humilis* had been used previously in *Mentzelia* for a taxon treated by Urban & Gilg (1900) as a variety of *M. pumila* Torr. & A. Gray. Subsequently, Darlington (1934) raised *M. pumila* var. *humilis* Urb. & Gilg of section *Bartonia* to the rank of species as *M. humilis*. Unfortunately, she also treated *A. humilis* and *M. humilis* as homotypic and incorrectly synonymized the name *Acrolasia humilis* under *Mentzelia humilis*.

When Glad (1976) named *M. thompsonii*, she did not mention *Acrolasia humilis*, a name that, nevertheless, could not have been transferred to *Mentzelia* because of Darlington's (1934) earlier elevation of *M. pumila* var. *humilis* to species status. Thus, *M. thompsonii* became the accepted name (in treatments recognizing *Trachyphytum* as a section of *Mentzelia*; e.g., Holmgren et al. 2005) for the species originally described as *Acrolasia*

FIG. 5. Lectotype of *Mentzelia humilis* (Urb. & Gilg) J. Darl. Specimen on right side of sheet is the lectotype (*C. Wright 214,* 1849) mounted with the non-type specimen (*H. N. Patterson s.n.*, 1875) on the left.

humilis. Subsequently, the nomenclatural combination *A. thompsonii* (Glad) W. A. Weber, was proposed to accommodate use of *Acrolasia* in treatments of the Rocky Mountain flora (Weber 1984). However, rediscovery of the original description and holotype of *A. humilis* led to synonymization of *A. thompsonii* and a return to the use of *A. humilis* in later treatments (Weber and Wittman 1992). The final taxonomic error occurred when Kartesz (1999) repeated Darlington's (1934) incorrect synonymy of *A. humilis* with *M. humilis* and went on to designate *M. thompsonii* and *A. thompsonii* as synonyms of *M. humilis*.

Our reconstruction of the taxonomic history of *M. thompsonii* indicates that a failure to consult type specimens has played a major role in the current taxonomic confusion surrounding this species. Even cursory examination of types of *M. thompsonii* and *M. humilis* (Figs. 4 and 5, respectively) reveals that these names do not represent the same taxon. Given the new evidence from cytology and molecular phylogenetics (Brokaw and Hufford 2010a) we hope that earlier taxonomic misinterpretations will be put to rest. *Mentzelia thompsonii* is a distinct species with a unique phylogenetic history and ecological niche,

and it is an important piece of the evolutionary puzzle that is *Mentzelia*.

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A NEW SPECIES OF *MENTZELIA* (LOASACEAE) FROM MONO COUNTY, CALIFORNIA

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ABSTRACT

A new species **Mentzelia monoensis** Brokaw and Hufford, endemic to Mono County, California, is described. **Mentzelia monoensis**, a hexaploid, is most similar to two widespread species, *M. montana* (Davidson) Davidson (tetraploid) and *M. albicaulis* (Douglas ex Hook.) Douglas ex Torr. & A. Gray (octoploid). The three species can be distinguished based on differences in floral bracts, fruits, and seeds. **Mentzelia monoensis** is commonly found in volcanic soils derived from the eruptions of Mono Craters and may exhibit edaphic specializations that limit its distribution.

Key Words: endemic, *Mentzelia*, Mono Craters, polyploidy, systematics.

Mentzelia L. section *Trachyphytum* Torr. & A. Gray (Loasaceae) is a monophyletic group of at least 22 species of annual plants occurring primarily in the western United States with greatest taxon richness in California (Darlington 1934; Zavortink 1966; Hufford et al. 2003; Brokaw and Hufford 2010a, b). The group is taxonomically difficult because of overlapping morphological variation among polyploid species (Zavortink 1966; Brokaw and Hufford 2010b). In her revision of the section, Zavortink (1966) noted a single hexaploid population in Mono County, California, which she dubbed "M. monoensis" but never validly published. In addition to the rarity of specimens available for study, confusion regarding the status of "M. monoensis" has been exacerbated by its similarity to other species in the section. However, recent molecular investigations (Brokaw and Hufford 2010b) have suggested a unique hybrid origin of "M. monoensis" and stimulated a deeper investigation of its form and distribution. That study indicated "M. monoensis" is morphologically distinct from other species of section *Trachyphytum* and endemic to Mono Co., California, where it is distributed throughout the Mono Craters region. In this paper, we describe "M. monoensis" as a new species of *Mentzelia*.

MATERIALS AND METHODS

During this study, we made field observations and inspected herbarium specimens of all North American species of *Mentzelia* section *Trachyphytum*. Zavortink (1966) based her original recommendation for recognition of 'M. monoensis' on specimens from the collection *Zavortink 2640* and chromosome counts from squashes of microsporocytes from the same population. We have located and included fifteen additional population of 'M. monoensis' in morphological comparisons with herbarium specimens, including an isotype (UC68802, *Hall 6577*), of *M. montana* (Davidson) Davidson. Morphological measurements were taken with digital calipers, using a dissecting microscope when necessary. Seed surface characters were assessed using scanning electron microscopy (SEM). Seeds of "M. monoensis" obtained from the herbarium specimens WS376107 (*Zavortink 2640*) and WS375796 (*Brokaw 368*), and seeds of *M. montana* from the herbarium specimens LA100619 (*Zavortink 2586*) and WS367986 (*Brokaw 72*), were mounted on metal stubs and coated in gold prior to imaging. Seeds were examined at 12 kV using a Hitachi S-570 scanning electron microscope and micrographs were made at 70×, 80×, and 750×. Locality data for populations were gathered during fieldwork and from herbarium specimens. Latitude and longitude or UTM coordinates for new collections were made in the field using GPS (WGS 84 map datum).

TAXONOMY

Mentzelia monoensis J. M. Brokaw & L. Hufford, sp. nov. (Fig. 1). —TYPE: USA, California, Mono Co., along State Hwy 120, 8 mi E of U.S. 395, 16 June 2007, *J. M. Brokaw 367* (holotype: WS; isotypes: NY, UC, US).

Herba annua, 10–30 cm alta, erecta. Inflorescentia cymis; bractea integra viridia rarior basis albus. Calyx connatus, sepala 5, subulatus.

FIG. 1. Type specimen of *Mentzelia monoensis*. Scale bar = 10 cm.

Apopetalus, petala 5, usque 4.1 mm longa, apex luteus et basis aurantiacus. Stamina 10–30, usque 3 mm longa. Ovarium inferum, placentae 3 parietalibus; stylus usque 3.3 mm longa. Fructus capsularis, Seminum numerosus, testa colliculate.

Annual herbs, 10–30 cm tall; taprooted. Shoots densely pubescent throughout (except as noted) with both needle-like and glochidiate trichomes; needle-like trichomes with pointed apex and erect barbs arranged in many whorls along the needle shaft; glochidiate trichomes usually with apical and several other whorls of recurved barbs along the stalk. Stem erect; axillary branches ascending, straight to curved upward; stem epidermis light tan to salmon-colored, moderately pubescent. Leaves alternate, pubescent with greater density of trichomes on abaxial surface, trichomes needle-like and glochidiate; needle-like trichomes often with pustulose bases; basal rosette leaves 2.1–86 × 0.3–10 mm, linear, all sessile or some appearing petiolate due to narrowed lamina at base, often not persisting to maturity, margins entire to 12-lobed on distal half, lobes nearly opposite, regular, 1.0–3.8 mm long with rounded apices; lower cauline leaves 6.2–55 × 2.0–7.9 mm, linear to lanceolate, sessile to appearing petiolate, margins entire to 12-lobed on distal half, lobes nearly opposite, regular, 0.5–3.5 mm long with rounded to acute apices; upper cauline leaves up to 34.2 × 8.5 mm, linear to ovate, sessile to clasping, margins usually entire. Inflorescence cymose, bract subtending inferior ovary 1.5–4.5 × 0.4–2.3 mm, entire, green (rarely with inconspicuously white base). Flowers epigynous; bearing a hypanthium at the distal end of the ovary on which the calyx, corolla, and androecium are inserted. Calyx basally connate, five triangular lobes, lobes 1.9–3.1 × 0.8–1.1 mm, apices acute to attenuate, margins entire, pubescent; trichomes like those of leaves. Petals five, distinct, 2.1–4.1 × 1.9–3.1 mm, obovate, with yellow apex and orange base, glabrous, apex retuse to rounded with several trichomes at midvein; trichomes with erect barbs arranged in many whorls, apex needle-like, base not pustulose. Androecium yellow; stamens ca. 10–30, ca. 2–3 mm, those of inner whorls shorter than outer whorls; filaments all filiform. glabrous; anther epidermis papillate. Gynoecium 3-carpellate; ovaries inferior, placentae 3, parietal; styles 2.2–3.3 mm long, glabrous; stigmas three, lobes appressed, papillate. Fruit a capsule, 6–15 × 2–3 mm, cylindrical to clavate and tapering near base, erect to curved less than 20°, opening apically, usually without prominent longitudinal ribs, pubescent; trichomes like those of leaves. Seeds ca. 1 × 1 mm, ca. 5–30 per capsule, in more than one row above mid-fruit; seeds above mid-fruit irregular-rounded; seeds below mid-fruit occasionally trigonal prisms with grooves along longitudinal edges; seed coat tan, colliculate under 10× magnification, domes less than 1/2 as tall as wide; seed coat cells 25–75 μm wide (smaller near hilum), with straight anticlinal walls.

Chromosome number: $2n = 54$ (Zavortink 1966).

Phenology: *Mentzelia monoensis* begins flowering at lowest elevations in late May and continues through late July. Plants bearing both flowers and ripe capsules with mature seeds are most common in early to middle July. By early August, most plants have senesced and quickly disintegrate.

Etymology: This species is named for the Mono Craters region and Mono Co., California, to which it appears to be endemic. We suggest the common name Mono Craters blazingstar.

Distribution: Populations of *M. monoensis* occur primarily on course pumice soils and disturbed sites near the Mono Craters of Mono Co., California, at 2008–2482 m elevation. They are associated especially with antelope bitterbrush and Jeffrey pine communities and barren pumice slopes.

Representative specimens. USA. CALIFORNIA. **Mono Co.**: Mono Craters, along State Hwy 120 about 8 mi. E of U.S. Hwy 395, *Thompson 1696* (LA); along State 120, 8 mi. E of U.S. 395, *J. Zavortink 2640* (RSA, WS); CA State Hwy 120 N of Mono Mills in barren pumice valley, UTM 11S 4197240mN 325071mE, *Brokaw 368* (ACU, WS); CA State Hwy 120 NE of Crater Mountain, UTM 11S 4197256mN 325051mE, *Brokaw 519* (WS); on North Crater S of Mono Lake, UTM 11S 4199798mN 319945mE, *Brokaw 520* (WS); in the East Craters Sand Flat, S of Crater Mountain and E of Punch Bowl, 37.82022°N, 119.00142°W, *Brokaw 547* (ACU, WS); along CA State Hwy 120 W of its junction with Sage Hen Meadows Rd, 37.89023°N, 118.86738°W, *Brokaw 554* (ACU, WS); at the junction of U.S. Hwy 395 and CA State Hwy 120, 37.88666°N, 119.09028°W, *Brokaw 558* (ACU, WS); E of U.S. Hwy 395 S of its junction with West Portal Rd, 37.86439°N, 119.08492°W, *Brokaw 559* (ACU, WS); along CA State Hwy 120 SW of Granite Mountain, 37.89059°N, 118.78930°W, *Brokaw 560* (ACU, WS); along CA State Hwy 120 E of Granite Mountain near its junction with Dobie Meadows Rd (Rd 3027), 37.92131°N, 118.70541°W, *Brokaw 561* (ACU, WS); along CA State Hwy 120 E of Granite Mountain N of Indian Meadows, 37.91792°N, 118.71134°W, *Brokaw 562* (ACU, WS); E of Lake Crowley along Benton Crossing Rd N of Round Mountain, 37.63795°N, 118.63873°W, *Brokaw 566* (ACU, WS); along West Portal Rd E of U.S. Hwy 395, 37.84638°N, 119.06704°W, *Brokaw 571* (WS); along West Portal Rd E of U.S. Hwy 395, 37.84789°N, 119.06490°W, *Brokaw 572* (ACU, WS); along CA State Hwy 167 at Wilson Creek,

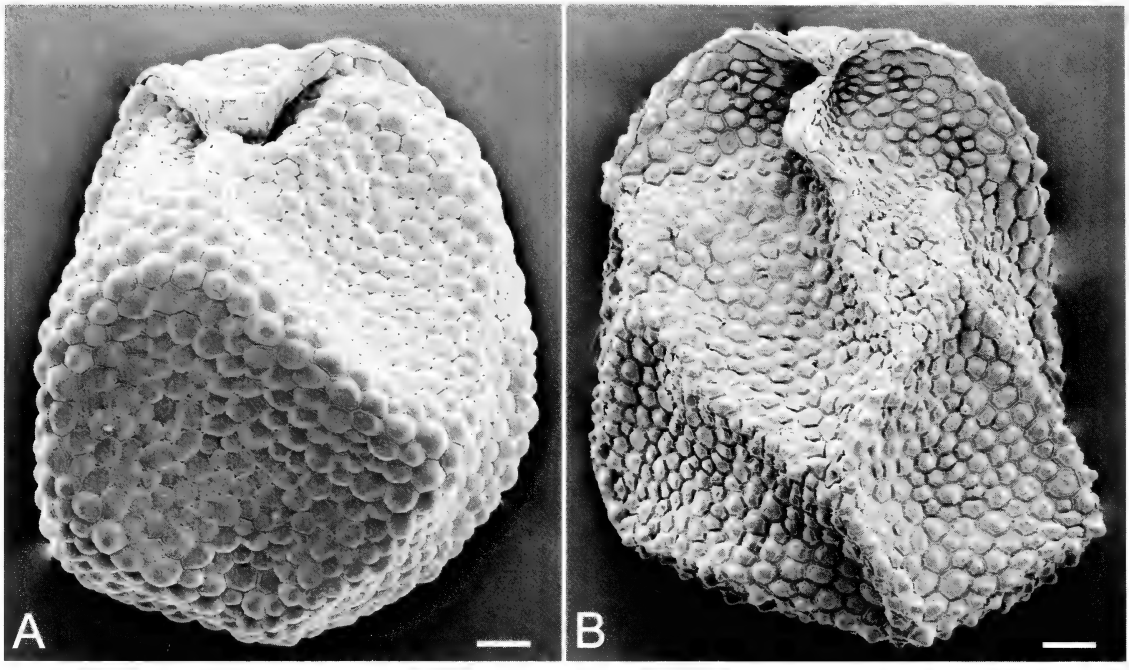

FIG. 2. Scanning electron micrographs showing variation in seed coat cell shape in: A. *Mentzelia monoensis* and B. *M. montana*. Scale bars = 100 μm.

38.05379°N, 119.12708°W, *Brokaw 573* (ACU, WS).

DISCUSSION

Recent phylogenetic analyses suggest that *M. monoensis* has a unique allopolyploid origin (Brokaw and Hufford 2010b). *Mentzelia* section *Trachyphytum* is composed of two major clades, "Affines" and "Trachyphyta" (Zavortink 1966; Brokaw and Hufford 2010a). Most polyploid species in *Trachyphytum* appear to be allopolyploids derived from hybridizations within the "Trachyphyta" clade (Zavortink 1966; Brokaw and Hufford 2010b). The only exceptions are *M. dispersa* S. Watson of the "Affines" clade, which may be an autopolyploid complex, and *M. monoensis*, the only species exhibiting substantial genetic signal from both major clades (Brokaw and Hufford 2010b). One hypothesis of origin consistent with the molecular data is that *M. monoensis* was derived through allopolyploidization involving a diploid progenitor closely related to *M. dispersa* and a tetraploid progenitor closely related to *M. montana* (Brokaw and Hufford 2010b). Both *M. dispersa* and *M. montana* occur in the Mono Craters region.

Mentzelia montana and *M. congesta* Torr. & A. Gray (diploid) are the only species in section *Trachyphytum* that have been found to co-occur with *M. monoensis*, though *M. laevicaulis* (Douglas ex Hook.) Torr. & A. Gray of section *Bartonia* Torr. & A. Gray has also been observed in proximity. Co-occurrence with *M. montana* is of particular concern because some populations of *M. monoensis* and *M. montana* can only be distinguished with difficulty. The bracts of *M. monoensis* are always entire and usually fully green, and those of *M. montana* are usually lobed with a white base. However, both species may exhibit entire floral bracts with whitish bases, though the white is more prominent in *M. montana*. In sympatric populations the grayish-green hue in leaves of *M. monoensis* may be also be apparent compared to the lighter green in leaves of *M. montana*. The two species can be most reliably distinguished when mature seeds are compared under 10–20× magnification. *Mentzelia monoensis* has a tan colored seed coat composed of cells that are rounded, appearing as shallow domes (Fig. 2A). In contrast, *M. montana* has a mottled seed coat with cells that stand out as rough, pointed knobs along edges of the seed (Fig. 2B).

Likewise, despite superficial similarity of the plants, the seeds of *M. monoensis* can be distinguished from those of *M. albicaulis* (Douglas ex Hook.) Douglas ex Torr. & A. Gray (octoploid), which also have a mottled seed coat but have cells that project from the surface to an even greater extent than those of *M. montana*, giving a distinctly rough appearance to the seeds. The bracts of *M. albicaulis* exhibit approximately the same range of form and color found in *M. monoensis*. However, *M. albicaulis* is distinct from both *M. monoensis* and *M. montana* in its

FIG. 3. Habit of *Mentzelia monoensis*. East Craters Sand Flat, Mono Co., California.

fruit shapes and distribution. Unlike the short, erect fruits of *M. monoensis* and *M. montana*, mature specimens of *M. albicaulis* usually have at least some long, recurved fruits greater than 15 mm and curved between 90° and 180°. In Mono Co. both *M. monoensis* and *M. montana* occur above 2000 m, and *M. albicaulis* occurs below 2000 m.

Like *M. monoensis*, *M. nitens* Greene has entire, green bracts and shallowly domed seed coat cells. However, like *M. albicaulis*, it has long, curved fruits and only occurs below 2000 m. *Mentzelia nitens* is distinguished from other species from section *Trachyphytum* in Mono Co. by its large petals ($\geq$ 8 mm).

The only hexaploid species of *Mentzelia* in northeastern California other than *M. monoensis* is *M. veatchiana* Kellogg. *Mentzelia veatchiana* is more robust than *M. monoensis* with larger flowers (styles $>$ 3.5 mm) and lobed bracts. Further, many populations of *M. veatchiana* exhibit orange petals with red bases, unlike *M. monoensis* and all other species in *Trachyphytum* that occur in eastern California.

Despite the difficulty of identifying some populations of *M. monoensis*, this species is an

important endemic component of the Mono Craters flora. Although often difficult to distinguish morphologically, unrecognized polyploid species may result in substantial underestimates of diversity (Soltis et al. 2007). For example, recently published floras have lumped diploid, tetraploid, hexaploid, and octoploid cytotypes as the single taxon, *M. albicaulis* (Holmgren et al. 2005), and no morphological characters to distinguish diploid, tetraploid, and octoploid cytotypes of *M. dispersa* have been identified (Brokaw and Hufford 2010a).

The occurrence *M. monoensis* in a region largely composed of volcanic substrates suggests that the establishment of populations after polyploidization may have been associated with new edaphic specializations. *Mentzelia monoensis* has been found in habitats ranging from pumice soils and gravels in open barrens, *Purshia* scrub, and pine forests (Fig. 3) to disturbed sites along roadsides. Similar to other species in *Mentzelia* (Prigge 1986), *M. monoensis* appears to avoid competition in productive communities through colonization of disturbed and/or stressful habitats. Further investigation of possible substrate specificity is needed in order to better understand the function of *M. monoensis* in the Mono Craters plant communities.

Identification Key for *Mentzelia* Section *Trachyphytum* in Mono County, California

1. Seeds in one row above mid-fruit, all trigonal prisms with grooves along longitudinal edges . . . . *M. dispersa*
1′ Seeds in more than one row above mid-fruit; seeds above mid-fruit irregular-rounded to -angular, seeds below mid-fruit occasionally trigonal prisms with grooves along longitudinal edges
    2. Floral bracts usually entire, green only
        3. Petals greater than or equal to 8 mm . . . . . *M. nitens*
        3′ Petals less than 8 mm
            4. Longest mature fruits usually greater than 15 mm, curved less than 180°; seeds tan and moderate- to dense-mottled brown to black in age; seed coat cells pointed or domed, in age greater than 1/2 tall as wide on seed surface edges; below 2000 m . . . . . *M. albicaulis* (in part)
            4′ Longest mature fruits less than 15 mm, curved less than 20°; seeds tan, not or sparse-mottled in age; seed coat cells flat-surfaced to domed, in age less than 1/2 tall as wide on seed surface edges; 2000–2500 m . . . . . *M. monoensis* (in part)
    2′ Floral bracts toothed to lobed or floral bracts entire with white base and green margin
        5. Floral bracts conspicuous, concealing fruits, mostly white with green fringe . . . . . *M. congesta*
        5′ Floral bracts not conspicuous, not concealing fruits, entirely green or mostly green with white below middle only
            6. Petals with orange to yellow apex and red to orange base; styles greater than or equal to 3.5 mm; longest mature fruits usually greater than 15 mm. . . . . *M. veatchiana*
            6′ Petals with yellow apex and orange base; styles less than 3.5 mm; longest mature fruits usually greater than 10 mm
                7. Floral bracts 3-lobed to entire; longest mature fruits usually greater than 15 mm, usually curved less than 180°; below 2000 m . . . . . *M. albicaulis* (in part)
                7′ Floral bracts 7-lobed to entire; longest mature fruits usually less than 15 mm, curved less than 45°; above 2000 m
                    8. Floral bracts 7-lobed to entire, white base usually conspicuous; seeds tan and moderate- to dense-mottled brown to black in age; seed coat cells pointed, in age some greater than 1/2 tall as wide on seed surface edges; above 2000 m . . . *M. montana*
                    8′ Floral bracts entire, white base inconspicuous; seeds tan, not or sparse-mottled in age; seed coat cells flat-surfaced to domed, in age less than 1/2 tall as wide on seed surface edges; 2000–2500 m . . . . . *M. monoensis* (in part)

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NOTEWORTHY COLLECTION

ARIZONA

ARTEMISIA PYGMAEA A. Gray (ASTERACEAE).—Mohave Co., flatland, E of road to Antelope Valley, 5000 ft, T40N R4W S36, 10 Sept 1977, *S. Clark 3700* (BLM Arizona Strip District Herbarium, St. George, UT); ca 10 mi SW of Fredonia and 4 mi S of Hwy 389 along the Mt Trumbull Rd, small shrubs local on white Moenkopi badland exposure, growing with *Artemisia tridentata* and *Eriogonum corymbosum*, T40N R4 W S36 NENW, 22 Sept 1999, *John L. Anderson 99-30* (ASU); Mt. Trumbull Loop, 350731 4077310N, *Clifton 41711* (Clifton private herbarium). Apache Co., Red Valley, ca 3 mi NW of jct. between Navajo Rtes 12 and 134, fine red clay soils of Chinle formation, occasional with *Artemisia bigelovii*, *Yucca angustissima*, *Sitanion hystrix*, *Juniperus monosperma*, 12S 674218 3988143N, 2206 m (7280 ft), 12 June 2003, *Roth 1600* (ASC, SJC).

Previous knowledge. Artemisia pygmaea extends from northern Nevada, Utah and Colorado south to northern New Mexico and northern Arizona. In Arizona it has only been known from one locality south of Fredonia where it has been collected several times since 1945 (*Darrow 3006* [ARIZ]).

Significance. The above collections document the second and third localities of *Artemisia pygmaea* in Arizona. *Anderson 99-30* is only ca 16 km (10 mi) from the previous known Arizona locality, but *Roth 1600* is ca 320 km (200 mi) east, near the New Mexico state line. Its importance is as an indicator of rare plant habitat since "...it occurs in peculiar edaphic habitats...where it is often a component of communities that support rare plant species" (Welsh et al. 2003). At the Fredonia locality *Artemisia pygmaea* occurs with *Pediocactus sileri* Gray, a Moenkopi Formation endemic.

BURSERA MICROPHYLLA A. Gray (BURSERACEAE).—La Paz Co., Harquahala Mts., foothills on the S side, ca 4 mi NE of Salome Hwy. below Socorro Peak, small shrubs (< 1 m tall) growing on steep south-facing hillsides of Paleozoic gray limestone with *Parkinsonia microphylla*, *Carnegia gigantea*, *Fouquieria splendens*, *Ferocactus cylindraceus*, *Opuntia bigelovii*, *Encelia farinosa*, *Hyptis emoryi*, T5N R11W S30 center, 2100 ft (640 m) 19 Mar 2001, *John Anderson 2001-22 and Leanna Anderson* (ASU, ARIZ).

Previous knowledge. Bursera microphylla (elephant tree, torote blanco, copal) ranges throughout the Sonoran Desert from western Sonora and southern Baja California north to southern Arizona and disjunct in California in the Anza-Borrego Desert (Kearney and Peebles 1960; Felger 2000). In Arizona *Bursera microphylla* is known from approximately fifteen desert mountain ranges primarily just north of the Mexican border.

Significance. The Harquahala Mountains location represents the northernmost known occurrence of *Bursera microphylla*. This site is 120 km (75 mi) north of the nearest occurrence to the south in the Mohawk Mountains (*Salywon 547, 551* [ASU]), east of Yuma, Arizona, and 80 km (50 mi) northwest of the White Tanks Mountains (*Keil 4012, 5943, 6191* [ASU]), west of Phoenix, Arizona. The Harquahala Mountains *Bursera microphylla* plants are dwarf shrubs due to the harshness of the habitat at this northernmost location, but in the main part of their range in Mexico they are trees 2–6 m tall.

FUIRENA SIMPLEX Vahl (CYPERACEAE).—La Paz Co., Grapevine Springs on S side of the Santa Maria River, first spring E of Mine Spring, locally common at spring, with *Prosopis velutina*, *Salix gooddingii*, *Baccharis sergiloides*, *Vitis arizonica*, surrounding hills are Sonoran Desert, T11N R11 S21 SENE, 1400 ft (425 m), 18 Sept 1997, *John L. Anderson* 97-27 (ASU).

Previous knowledge. Fuirena simplex is widespread from the midwest (Kansas, Missouri, and Illinois) south through Mexico and the West Indies to northern South America (Kral 2003). Imdorf (1994) documented the first record of *Fuirena simplex* in Arizona. There it occurs at a spring in oak-juniper woodland at 4800 ft (1450 m) in the Sierra Ancha Mountains of east central Arizona.

Significance. The La Paz Co. record documents a second locality in Arizona and is 240 km (150 mi) west of the previous Arizona record. The Grapevine Springs site is also over 1000 m lower in elevation than the Sierra Ancha Mountains location. Its adjacent vegetative community, Sonoran Desertscrub, is very different from the Madrean Evergreen Woodland at the Sierra Anchas locality. The Grapevine Springs locale is also dissimilar from the usual habitats of *Fuirena simplex* described as "low open woods, savannas and prairies" (Kral 2003).

PHOLISTOMA MEMBRANACEUM (Benth.) Constance (HYDROPHYLLACEAE).—Mohave Co., in large wash about 0.1 mi n of junction of Bonelii Landing Rd 74 and Temple Bar Rd, 550 m, 24 March 2001, *Katherine Birgy s.n., Seth Thompson and Elizabeth Powell* (ARIZ, UNLV); Wilson Ridge, W side, canyon N of LMNRA Rd 64 (Boundary Mine Rd), Mohave Desert, sandy wash bottom along base of narrow canyon, with *Keckiella antirrhinoides* ssp. *microphylla*, *Viguiera deltoidea*, *Salazaria mexicana*, *Brickellia californica*, *Bebbia juncea*, *Penstemon bicolor*, T30N R22W S26E, 3200 ft (970 m), 2 Apr 2003, *John L. Anderson 2003-17* (ASU, ARIZ); Petroglyph Wash, 721059 3993362N, *Clifton 43456* (Clifton private herbarium).

Previous knowledge. Pholistoma membranaceum is widespread through the southern two thirds of California from the coast, foothills, and desert in a variety of habitats below 4750 m. It also extends into Baja California.

Significance. The above collections, all from the Wilson Ridge area, represent first records for Arizona of *Pholistima membranaceum*. Wilson Ridge, part of the Black Mountains, is directly east of the Colorado River and Nevada.

PULICARIA PALUDOSA Link (ASTERACEAE).—Yuma Co., Mitry Lake State Wildlife Area, 0.5 mi E of AZ/CA stateline and 6.2 mi W of Hwy 95 on Imperial Dam Rd, roadside in damp soil with *Phragmites*, *Typha*, *Pluchea*, *Polypogon*, T6S R21W S31NW, 11S 0737901 3639858, 176 ft (53 m),15 July 2010, *John L. Anderson 2010-14* (ASU). La Paz Co., Cibola National Wildlife Refuge, Island Unit (Unit 3), 0.5 mi W of Colorado River on Island Road, edge of flooded field with *Cynodon dactylon*, *Typha*, *Salix gooddingii*, *Prosopis*, 11S 0715264 3687034, 200 ft (60 m), 30 Sept 2010, *John L. Anderson 2010-26* (ASU); Parker, east bank of Colorado River just

downstream from Hwy 62 Bridge between Parker, AZ, and Earp, CA, densely vegetated mudflat with *Echinochloa lemmonii*, *Cynodon dactylon*, *Arundo donax*, *Typha*, *Pluchea purpurascens*, *Baccharis salicifolia*, 11S 0749065 3782807, 350 ft (105 m), 30 Sept 2010, *John L. Anderson 2010-27* (ASU).

Previous knowledge. *Pulicaria paludosa* is a native of the Mediterranean region of Portugal and Spain, reflected in its common name, Spanish false fleabane. It is introduced in California (Preston 2006) where it was first collected in 1946 (Orange Co., Rancho Santa Ana, Santa Ana River Canyon, moist sandy bank, *Munz 11554* [RSA], Preston 2006) and first documented in 1963 Raven (1963). In California, *Pulicaria paludosa* is primarily known from coastal southern California with a Mediterranean climate similar to its native habitat; it also occurs in the Palm Springs area and all along the Colorado River adjacent to Arizona: Squaw Lake, Imperial Co., *Bell 230* (UCR); Blythe, Riverside Co., *Ballmer s.n.* (UCR); Earp, San Bernardino Co., *McGaugh s.n.* (UCR); Whipple Mts, San Bernardino Co., *DeGroot et al 3348, 4367, 4382* (RSA). The collection *McLaughlin 4318* has a label location of "Sand island in Colorado River, near outlet of Taylor Lake" but with different counties on different duplicates at different herbaria: Imperial/Yuma Cos. (RSA), Imperial Co. (UCR), and Yuma Co. (ARIZ). S. McLaughlin (Univ. of Arizona, personal communication) stated that the collection *McLaughlin 4318* was from the California side of the Colorado River.

Significance. The above collections extend the range of *Pulicaria paludosa* east across the Colorado River from California into Arizona and represent the first records for Arizona. Though, as noted above, *Anderson 2010-26* was actually collected west of the Colorado River but still in Arizona where the CA/AZ state line follows an old meander which is west of the present course of the Colorado River.

Purshia glandulosa Curran (ROSACEAE).—Mohave Co., near Whitney Pass, gravelly, sandy loam, locally common, with *Yucca brevifolia*, *Y. baccata*, *Thamnosma*, *Encelia*, *Hymenoclea*, 3925 ft (1190 m), 22 Apr 1980, *Ralph Gierisch 4714* (ARIZ, ASC, ASU); Black Mountains, ca 7 air mi N of Union Pass, near radio facility and ca 2 mi N of Air Ranch, small shrubs growing on light-colored volcanic tuff (and extending onto adjacent rhyolitic hillsides), associated species include *Juniperus californica*, *Coleogyne ramosissima*, *Ericameria linearifolia*, *Salazaria mexicana*, *Salvia dorii*, *Cylindropuntia acanthocarpa*, T 22N R20W S2 SWSW, 4100 ft (1242 m), 26 Apr 1994, *John L. Anderson 94-5* (ASU).

Previous knowledge. *Purshia glandulosa* is not included in Arizona botanical references (Kearney and Peebles 1960; Shreve and Wiggins 1964; McDougal 1973; Lehr 1978; Benson and Darrow 1981) as part of the Arizona flora. Several floras of adjacent states do include Arizona in its range (Munz 1973; Hickman 1993; Cronquist et al. 1997; Welsh et al. 2003).

Significance. The above collections document the occurrence of *Purshia glandulosa* in Arizona. Mature fruits are needed in collections to make a positive identification of *Purshia glandulosa* because it resembles *P. stansburiana* vegetatively. Early season collections from other localities farther east in Mohave Co., Arizona, Quail Canyon (21 Apr 2000 *Higgins* (NY Accession Number 848039) and Cedar Pockets (2 May 2000 *Higgins* (NY Accession Number 848038), were identified as *Purshia glandulosa*. The author visited these localities on Aug 30 and Sept 1, 2010, respectively, and found the *Purshia* plants present to be *P. stansburiana*, having the multiple plumose-tailed achenes of *P. stansburiana*, not the single non-plumose achene of *P. glandulosa*. In Arizona *Purshia glandulosa* is a peripheral species of limited distribution, present only in the westernmost mountains in Mohave Co, Arizona, the Black Mountains (*Anderson 94-5*) and the Virgin Mountains (*Gierisch 4714*), adjacent to Nevada; nonetheless, it is a welcome addition to the Arizona flora. As Lester Rowntree (1939) said, "Although there is a great deal of *Purshia glandulosa* growing with Desert Artemisia on the mountains slopes bordering the desert, it seems never to be tiresome."

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NOTEWORTHY COLLECTION

CALIFORNIA

POLEMONIUM CARNEUM A. Gray (POLEMONIACEAE).—Siskiyou Co., Klamath National Forest, W of Yreka, uphill from USFS road 44N33, 1.1 air mi SE of Deadwood historical marker, 41°42.217′N, 122°47.645′W, 1332 m. Indian Creek Baldy quadrangle, T45N, R08W, Sec. 32, plants growing along a seasonal watercourse on a north-facing slope, with *Pseudotsuga menziesii*, *Isatis tinctoria*, *Berberis aquifolium* var. *repens*, *Amelanchier* sp., *Symphoricarpos* sp., *Agastache* sp., *Prunus virginiana*, *Ceanothus integerrimus*, *Lathyrus sulphureus*, the plants extended up the creek for 8 m, plants were ascending with multiple stems arising close together and almost a half meter tall, 25 June 2010, *Rebecca Stubbs 10 (SFSU, CAS)*.

A few additional plants were found extending E from the ravine, and there was a single plant growing at a lower switchback south of the larger population. D. Reed originally collected from this location in 1982 and deposited the specimen in the Scott River Ranger District of Klamath National Forest (CNDDB 2010).

Previous knowledge. *Polemonium carneum* had been found in the late 1800's and early 1900's as far south as the San Francisco Bay area. In the Consortium of California Herbaria the most recent collection was from 1950 with no other collections being deposited in the last six decades. The majority of the collections were made coastward. *Polemonium carneum* is common in Oregon and state-threatened in Washington.

Significance. Second report since 1950, the first by Reed in 1982 (CNDDB 2010). There was concern that *Polemonium carneum* had been extirpated from California.

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IN MEMORIAL

ISABELLE I. TAVARES 1921–2011

Dr. Isabelle Tavares died on May 21, 2011. Dr. Tavares had been associated with the University Herbarium for 59 years. Her academic career began at City College of Los Angeles, was interrupted by service in the Women's Army Corps during World War II, and continued at Berkeley after the War. She received B.A., M.A., and Ph.D. degrees from the University of California. For her doctoral dissertation, conducted under the guidance of Professor Lee Bonar, she investigated the Laboulbeniales, an order of minute fungi that parasitize insects. This research continued after her dissertation and eventually resulted in her magnum opus: *The Laboulbeniales*, published in 1985. She began working in the University Herbarium while still in graduate school, and continued uninterruptedly until long past her retirement in 1993. She curated the fungi (including lichens) and the bryophytes, and participated in all day-to-day operations of the Herbarium. Curating lichens led her to her second major research interest: the taxonomy of *Usnea*, a widespread and notoriously taxonomically difficult genus of lichens. Dr. Tavares was a founding member of the California Lichen Society, and an active promoter of California lichenology. Her involvement with the California Botanical Society was extensive, and she made major editorial contributions to *Madroño* over a period of many years. She was President of the Society from 1983–1984.

—Richard L. Moe, Collections Manager, University and Jepson Herbaria, University of California, Berkeley

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